SPATIAL AND TROPHIC BIOMONITORING OF TRACE METALS IN AQUATIC ENVIRONMENTS FOLLOWING THE MT. POLLEY MINE TAILINGS SPILL

by

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ABSTRACT

A large (~25 M m^3) copper-gold tailings spill occurred in 2014 at the Mt. Polley Mine, which released solid and liquid waste and caused a debris flow into nearby Polley Lake and Quesnel Lake. This study examined the spatial and trophic patterns of trace elements in water bodies impacted by the spill between April and November 2016. Concentrations of trace metals in biofilm, invertebrates, sediments, and water were measured and analyzed using DGT and ICP-MS. Trophic positioning of sampled organisms was established using invertebrate functional feeding groups, and stable isotope ratios. Spatial results indicated that copper and vanadium concentrations in biofilm and invertebrates at impacted environments regress significantly with distance to the spill. Trophic results show evidence of copper bioaccumulation and selenium trophic magnification in biofilm and invertebrates from impacted waters. These effects appear to have seasonal variations, with stronger trophic and spatial relationships during spring overturn of Quesnel Lake.

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ACRONYMS

AFDM – Ash Free Dry Mass

AMD – Acid Mine Drainage

BC – British Columbia

BMF – Biomagnification Factor

BLM – Biotic Ligand Model

CCME – Canadian Council of Ministers of the Environment

COSEWIC – Committee on the Status of Endangered Wildlife in Canada

CPUE – Catch Per Unit Effort

CTD – Conductivity Temperature Depth Sonde

DFO – Department of Fisheries and Oceans Canada

EPS – Extracellular Polymeric Substances

FIAM – Free Ion Activity Model

HCL – Hydrochloric Acid

ICP-MS – Inductively Coupled Plasma Mass Spectrometry

IDZ – Initial Dilution Zone

IRMS – Isotope Ratio Mass Spectrometry

MOE – Ministry of the Environment and Climate Change Strategy

MPMC – Mount Polley Mining Corporation

PEL – Probable Effect Level

PPM – Parts Per Million

PPB – Parts Per Billion

QRRC – Quesnel River Research Centre

TF – Transference Factor

TSF – Tailings Storage Facility

ELEMENTAL SYMBOLS

Ag – Silver

Au – Gold

As – Arsenic

Cd – Cadmium

Cr – Chromium

C – Carbon

Cu – Copper

Fe – Iron

Hg – Mercury

Mn – Manganese

N – Nitrogen

Ni – Nickel

Pb – Lead

Se – Selenium

V – Vanadium

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1 INTRODUCTION

1.1 BACKGROUND

Quesnel Lake is an important aquatic system supporting the health and biodiversity of fisheries in British Columbia, Canada. Peak years have seen > 1 million sockeye salmon (*Oncorhynchus nerka*) pass through the lake on their way to headwater spawning habitat, and Quesnel Lake itself hosts coho salmon (*Oncorhynchus kisutch*) and bull trout (*Salvelinus confluentus*) populations which are listed as species at risk by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2012, 2016). In 2014, Quesnel Lake was impacted by one of the largest mine spills in Canadian history, the Mt. Polley Mine tailings breach. This event released ~25 M m^3 of material (solids and liquids) into the landscape (Morgenstern et al., 2015). From this initial release, ~20 M m^3 of material, including mine waste, construction material, impoundment water, and material scoured along the receiving waterway (Hazeltine Creek), was delivered to Quesnel Lake (MPMC, 2015a). A stretch of the West Basin benthic region, approximately 5.5 x 0.5 km in surface area, was covered by a layer of spill and scour material (MPMC, 2015b), although more recent data suggest that the area covered by this material may be much larger. Spilled tailings were determined to be enriched with arsenic (As), copper (Cu), iron (Fe), and manganese (Mn) in excess of the Canadian sediment quality guidelines for the protection of aquatic life (CCME, 2018; MPMC, 2015a), but the biotic effects of the spill are largely unknown.

The magnitude, extent, and distribution of spill material in Quesnel Lake have been reported (Morgenstern et al., 2015; MPMC, 2015c), yet a gap exists in understanding the biochemical effects of mine-associated metals (Petticrew et al., 2015). Many trace metals deposited into Quesnel Lake have the potential to cause deleterious impacts to the aquatic community (Bradl, 2005). A direct way to assess the bioavailability and bioaccumulation of these contaminants in aquatic systems is by measuring concentrations within organisms (Zhou et al., 2008). Aquatic biofilm – mixtures of bacteria, fungi, algae, and other microbes in a matrix of secreted polymeric substances – is a potential pathway for metals into the food web, which has been successfully used to monitor the ecosystems impacts of industrial related metals worldwide (Morin et al., 2008; Tien and Chen, 2013), including in temperate nival environments of northwestern North America (Farag et al., 2007). It is the broad objective of this study to use biomonitoring, with biofilm as a key element, to better understand the distribution of mine-associated bioavailable metals in the landscape and food web of the Quesnel River Catchment.

1.2 QUESNEL LAKE AND CATCHMENT

Quesnel Lake is a narrow, deep and oligotrophic fjord lake located in the interior plateau of British Columbia (BC), Canada (Figure 1.1). It takes the shape of a "y" rotated 90° clockwise, with a West, North, and East Arm, and has a relatively small surface area (266 km^2) for its volume of 42 km^3. It has a mean depth of 157 m, and a maximum depth of 511 m (in the East Arm), making it the deepest fjord lake in the world (Laval et al., 2008). The West Arm of Quesnel Lake is separated into two parts by the shallow (35 m deep) Cariboo Island sill, which partially separates two basins, the West Basin and Main Basin (of the West Arm), which are partially hydrologically distinct. The West Basin makes up 8.6% of the lake's surface area, but only 2.3% of its volume (Laval et al., 2008). Based on average lake discharge to the Quesnel River of 131 m^3/s the average residence time of lake water is approximately 10 years for the lake as a whole (Laval et al., 2012), but only three months for the West Basin alone (Laval et al., 2008).

Although limnologic factors vary by season, Quesnel Lake tends to be thermally stratified in the period June-October, with the epilimnion depth slowly deepening throughout the summer and receding in the fall, and an ice-free seasonal average thermocline depth of ~9 m (Hume et al., 2005). The lake is dimictic, with overturn in both fall and spring, usually December and April (Laval et al., 2012). Lake water tends to be well buffered and slightly alkaline, with a pH ranging from 7.1-8.1, and an inter-annual average of ~7.6 (Hume et al., 2005). The lake is oligotrophic, with a mean total phosphorus concentration of ~2.7 µg/l, and relatively abundant total nitrate of 104 µg/l during spring overturn. Seasonal average chlorophyll concentrations are ~1.03 µg/l, with primary productivity likely to be phosphorus limited (Shortreed et al., 2001).

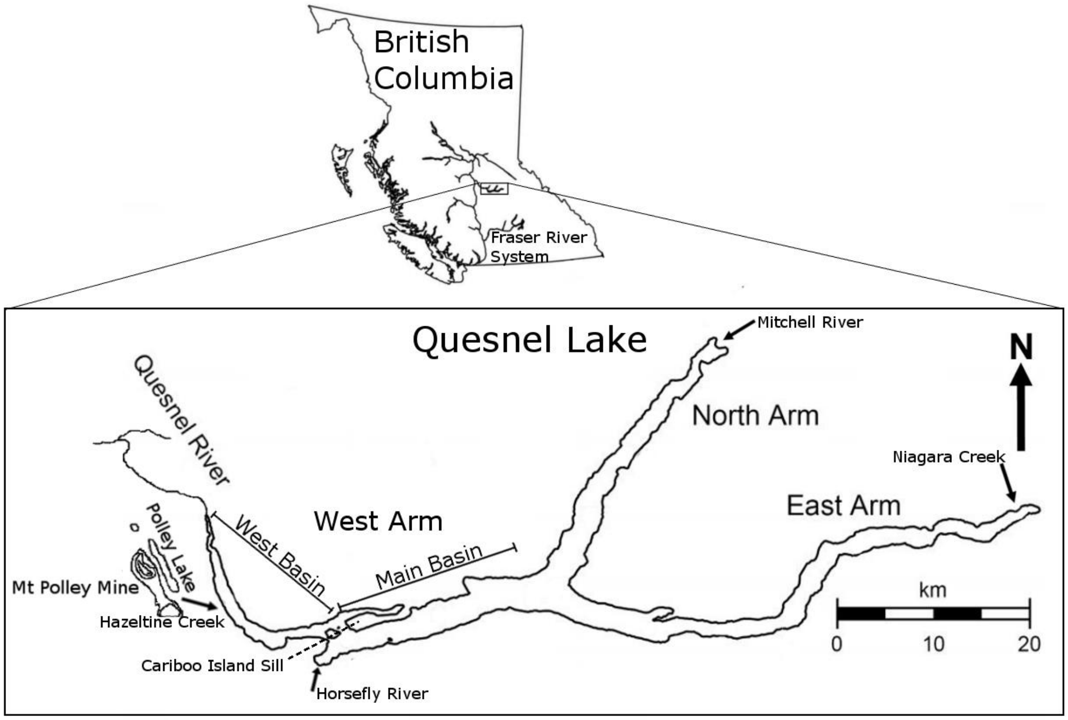

Figure 1.1 – Quesnel Lake and the Mt. Polley Mine located within the Fraser River system, British Columbia, Canada. The arms of the lake, the West and Main Basins, the Cariboo Island sill, and three main inflowing streams are shown. Adapted from Selbie (2014).

Quesnel Lake is part of the wider Quesnel River Catchment - sometimes called the Quesnel River Basin (Burford et al., 2009; Smith and Owens, 2014) - which covers an area of ~11,500 km^2 and is a tributary to the Fraser River system, the longest river and largest catchment in BC. The hydrologic regime of the Quesnel River Catchment is primarily nival, owing to the temperate climate and Cariboo Mountains which border the lake on its eastern side. Three main tributaries flow into Quesnel Lake. The Horsefly River is the largest of these, draining the interior plateau region to the south of Quesnel Lake, an area of ~2750 km^2 with an annual precipitation of 500-1000 mm/yr. Two large streams flow down from the Cariboo Mountains - Mitchell River and Niagara Creek - each draining ~600 km^2 including areas of ice fields and glaciers, with an average precipitation of 1500-2500 mm/yr (Potts, 2004). Many smaller creeks flow into the lake, including Hazeltine Creek, which flows from the south into the West Basin, and is notable because this is the path that spill materials took to enter Quesnel Lake. Flows in the catchment are typically lowest in February and March, before spiking rapidly in May during the freshet, peaking in June, and declining steadily into fall (Burford et al., 2009).

Water quality in the Quesnel River Catchment has historically been good. Localized industrial activity has taken place since approximately 1850, with numerous abandoned mines in the region (Clark et al., 2014). Forestry has occurred since the late 1900s, but predominantly in the western side of the catchment near the town of Quesnel (Smith and Owens, 2014). Community forestry has occurred with limited scale since 1990, and small-scale mines have operated in the region since its colonization in the 18th century. The first modern industrialized mine, the Mt. Polley Mine, opened in 1997. Land use by area in the Quesnel River Catchment is 62.8% natural forest, 31.9% forestry, 5% agriculture and 0.3% mining (Smith and Owens, 2014).

1.3 Geology and Mineral Processing at the Mt. Polley Mine

Quesnel Lake straddles two major geological bodies: the Omineca belt to the northeast, into which the East and North Arms of the lake protrude, and the Intermontane Belt to the southwest, which contains the Junction (the point where the three arms meet) and West Arm of the lake (Rees, 1987). More specifically, the west side of the lake and surrounding region are in a mineral-rich area called the Quesnel Terrane, which extends north and south parallel to the western slopes of the Canadian Rocky Mountains. The Quesnel Terrane contains numerous porphyry copper deposits, where slow-cooling igneous intrusions from the Mesozoic Era left small pockets (< 150 Mt) of high-grade ores containing 10 kg/t of copper (Cu) and 1g/t of silver (Ag) or gold (Au) (Friedman et al., 2014). The Mt. Polley deposit is an alkalinic intrusive complex, dating from the late Triassic, ~205 Ma ago. Copper and other trace metals occur primarily in sulfide minerals trapped in hydrothermal breccias, where volcanic activity interacted with ground water to form mineral-rich precipitates (Pass et al., 2014). In the upper layers of the deposit, these sulfide minerals were exposed to oxygen and weathered through oxidation, primarily into silicate minerals (MPMC, 2015a). When compared to global basalt rock averages, Mt. Polley Mine rock is enriched in many elements, including copper, arsenic and selenium (Figure 1.2).

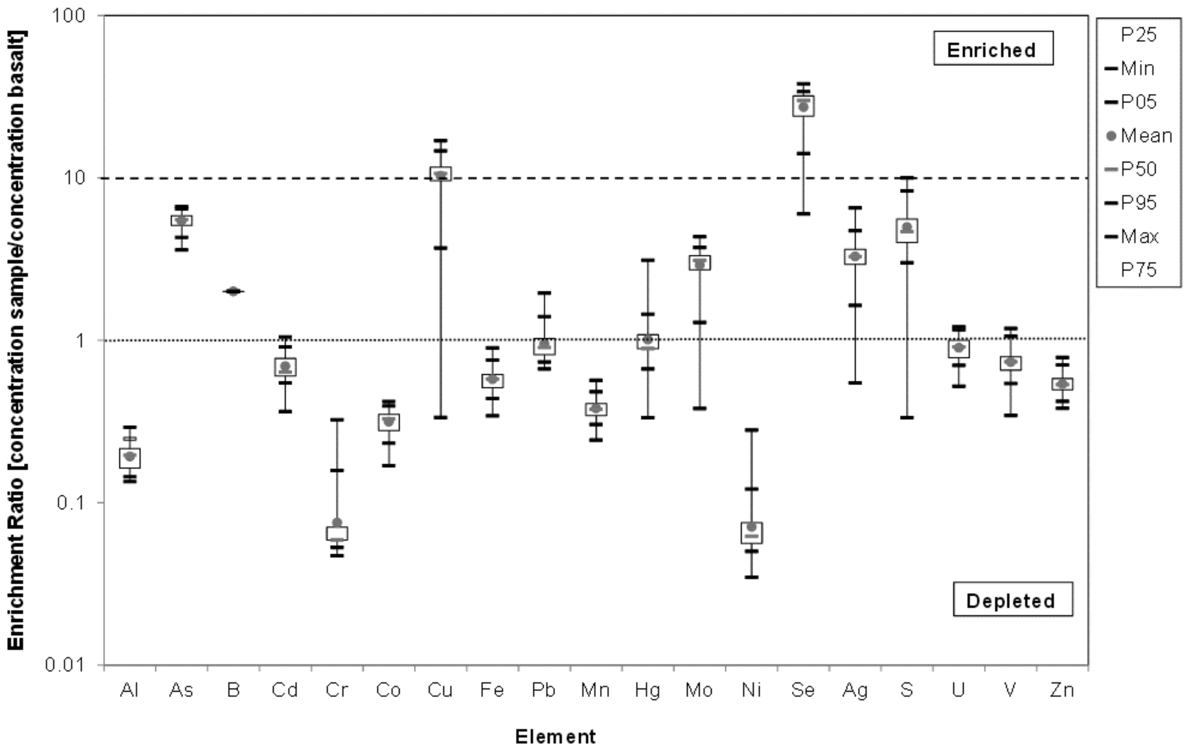

Figure 1.2 – Means and distributions of elemental enrichment values of Mt. Polley Mine tailings, compared to global basalt averages. The Y axis is logarithmic, with the horizontal dotted line indicating 10 times global basalt averages. Figure adapted from MPMC (2015a), based on guidelines from Price (1997)

The Mt. Polley Mine is located in a porphyritic intrusion which occurs between Polley Lake and Bootjack Lake, west of Quesnel Lake (Panteleyev et al., 1996). Ore processing involves blasting native rock deposits, followed by passing the material through three sequentially finer crushing and grinding mills to obtain a mean particle size of 50 µm (MPMC, 2015a). The milled rock is mixed into a slurry with water and reagents including hydrogen sulfide, methyl isobutyl carbinol, potassium amyl xanthate, calcium oxide, sodium hydrogen sulfide, and other undisclosed materials (MPMC, 2015a). These chemicals are designed to make copper-bearing sulfide minerals hydrophobic. The slurry is frothed by pumping air through the bottom of a tank, making the hydrophobic minerals float to the top where they are skimmed, while the undesired material sinks. “Tailings” refers specifically to the undesired leftover material from the froth flotation process. Tailings were delivered to the tailings storage facility, along with wastewater from milling and froth flotation.

Tailings sampled before the spill were known to have copper concentrations of ~1000 mg/kg, of which ~400 mg/kg was bound in organic molecules and ~25 mg/kg was loosely bound in reducible metals. Selenium concentrations were found to be in the range of ~1.5 - 2.0 mg/kg, ~0.25 mg/kg of which is exchangeable and ~0.25 mg/kg bound in reducible metals (MPMC, 2015a). Concentration alone is a poor predictor of trace metal bioavailability, but these high concentrations indicate a potential risk to aquatic life (Clearwater et al., 2002; Hamilton, 2004). As metals are much more easily extracted in acidic conditions, mineral leaching is often associated with acid mine drainage (AMD), usually caused by oxidation of sulfur minerals and accelerated by bacteria (Akcil and Koldas, 2006). At the Mt. Polley Mine, AMD has not been detected, probably due to the presence of abundant calcite (0.7 - 4.5%), a buffering mineral, and a relatively low sulfur content (0.1 - 0.3%) (MPMC, 2015a).

Humidity cell tests were performed on spilled tailings, meant to simulate open air leaching conditions with weekly wet and dry cycles (3 days dry, 3 days humid, 1 day leaching) as described in the provincial Acid Rock Drainage Prediction Manual (MEND, 1991). Leachate from these tests contained ~0.01 mg/l of copper and ~0.0075 mg/l of selenium (MPMC, 2015a).

Given the large quantity and surface area of material deposited into Quesnel Lake following the spill, metal enrichment is still possible, particularly in benthic areas in front of Hazeltine Creek, where the tailings material is the most concentrated.

1.4 TAILINGS IMPOUNDMENT BREACH

On August 4th, 2014, a catastrophic tailings breach occurred along the northern wall of the Mt. Polley Mine tailings storage facility (TSF). This area of the impoundment wall became over-pressurized at its foundation, breaching suddenly from the bottom up (Morgenstern et al., 2015). The breach released approximately 25 M m^3 of material into the surrounding landscape, consisting of approximately 20% tailings, 29% interstitial water, 48 % supernatant water, and 3% construction materials, which was delivered to the downstream environment within a few hours of the breach (MPMC, 2015c). This material flowed immediately into nearby Polley Lake, plugging the outflow and raising its level by 1.7 m (Petticrew et al. 2015). Tailings and debris continued down the path of least topographic resistance, the channel of Hazeltine Creek, scouring it from ~ 1.5 m wide to ~ 50 m wide, creating several barriers to fish passage and disrupting fish habitat in the process (MPMC, 2015c). Hazeltine Creek flows into Quesnel Lake, which rose 7.7 cm over an area of 266 km^2 as a result of the additional material, translating into a lake volume displacement of ~20 M m^3 (Petticrew et al., 2015). The material added to Quesnel Lake was composed of an estimated 68.8% tailings and interstitial water, 24.7% supernatant water, and 6.5% scoured soil and surficial material from Hazeltine Creek (MPMC, 2015c).

A sediment plume remained in the water column of the West Basin throughout the summer and early fall, with turbidity peaking at ~1000 NTU in deep waters in late August 2014 before gradually abating to ~100 NTU at the beginning of October 2014 (Petticrew et al., 2015).

Seiching in Quesnel Lake spread the plume up-lake into the Main Basin (i.e. over the Cariboo Island sill) as well as down-lake into the Quesnel River. The plume of sediment consisted of small particles (d_{50} ~1 μm), which stayed suspended for several months. The bulk of the tailings, particularly larger particles, settled onto the bottom of the West Basin as a benthic lens 3-10 m thick, extending over an area of approximately 2.75 km^2 (MPMC, 2015b).

The direct and immediate physical impact of the Mt. Polley spill is reasonably well documented, but the chemical and biological impacts are less so. Based on metal concentrations in the tailings, the most likely candidates for metal leaching and adverse environmental effects are copper and selenium, which have greatly elevated concentrations (Figure 1.2). In addition, manganese, iron, and arsenic were found to be enriched in spilled material beyond sediment quality guidelines for the protection of aquatic life (CCME, 2018; MPMC, 2015a). The environmental risk from trace metals in tailings is dependent on biogeochemistry and "scale specific processes of metals mobility" (Jianu et al., 2012). The spilled tailings material in Quesnel Lake is known to be small (mean particle size ~50 μm) (MPMC, 2016). The large surface area and volume of the spill over a landscape scale poses a substantial environmental threat. For an informed assessment of risk, further examination of biochemical mobility is needed, particularly relating to bioavailability and food web transfer. Due to the slow nature of elemental accumulation in food webs, these toxic impacts are not immediately apparent and may take many years to fully travel through the food web and have their concentrations equilibrate in aquatic communities (Morin et al., 2008).

Although unplanned and unprecedented, the tailings breach is not the only source of trace metals contamination from the Mt. Polley Mine into Quesnel Lake. At the time of writing, the Mt. Polley Mine is operating and discharging wastewater directly into Quesnel Lake, following their re-permitting and resumed operations on August 5th 2015 (Ministry of Environment, 2017). Instead of storing all of their wastewater in the tailings impoundment, as the mine had done prior to the breach in 2014, wastewater is being discharged at a maximum rate of 0.3 m^3/s through a diffuser into the hypolimnion of the West Basin of Quesnel Lake (MPMC, 2016). There are a number of water quality requirements included in the wastewater discharge permit, including maximal concentrations of arsenic, copper, iron and selenium. Although wastewater contains metals from the same mineral body and processing facility, this ongoing point-source of contaminants needs to be considered in interpreting metals data. High concentrations of trace metals in the aquatic environment may not be attributed to spilled tailings alone.

1.5 Biofilm and Invertebrate Biomonitoring

Biofilms, often called periphyton, are microbial microcosms with multiple trophic levels and high diversity (Lewandowski and Beyenal, 2013). The fundamental components are microbiota, usually a complex assemblage of bacteria, algae, fungi, diatoms, and other taxonomic groups, attached to a substrate and living within a matrix of extracellular polymeric substances (EPS) secreted by members of the assemblage (Figure 1.3). High molecular weight compounds, including polysaccharides, proteins, nucleic acids, lipids and other organic macromolecules, are collectively called EPS, which is secreted by microbes into the environment (Wingender et al., 1999). The EPS creates an adhesive aggregate, which retains water and protects biofilm constituents within a favorable microclimate, while usually allowing for the penetration of oxygen and essential nutrients along a gradient (Lewandowski and Beyenal, 2013). Many EPS compounds act as sorption sites for inorganic and organic molecules, a potential entry pathway for environmental contaminants to enter the food web (Hao et al., 2013).

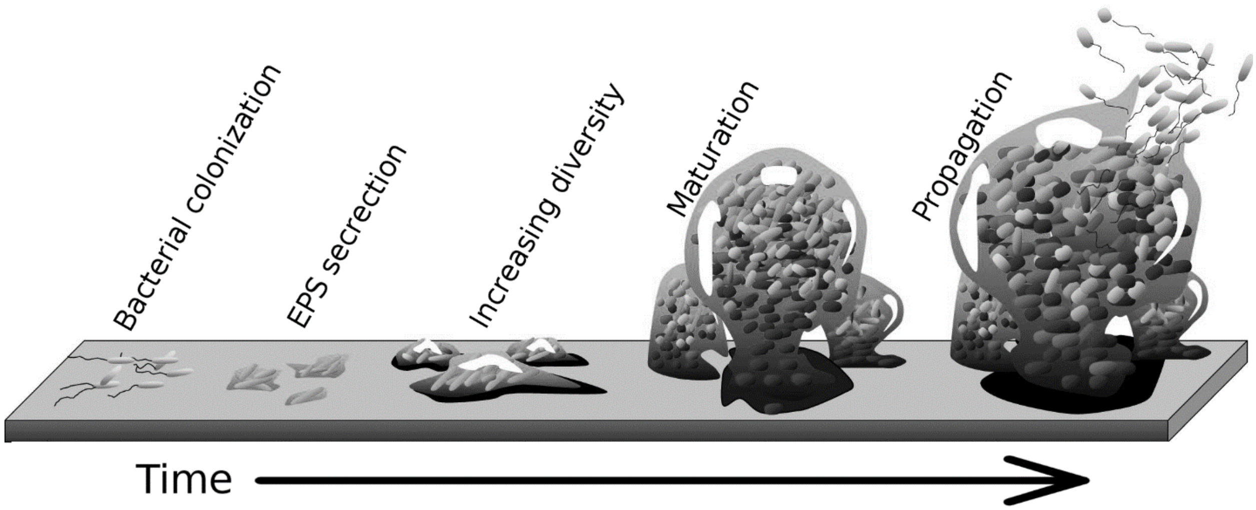

Figure 1.3 – Biofilm colonization, succession and propagation on a substrate. Bacterial colonizers attach and then excrete EPS, allowing a diversity of organisms to colonize. In a matter of weeks, parts of the biofilm will slough off, allowing the assemblage to propagate. Image adapted from Monroe (2007).

Biofilm forms the basis of benthic food webs and dominates primary productivity in many littoral areas of lakes and streams (Vadeboncoeur et al., 2008). In addition to their role as a primary food source, biofilms act as flocculants and traps for suspended particles (Eisenmann et al., 2001), affecting nutrient dynamics in freshwater environments. Of particular importance to the Quesnel River Catchment is the role played by biofilms in the delivery and retention of marine-derived nutrients transported by anadromous salmon (*Oncorhynchus spp.*) during their spawning cycle (Albers and Petticrew, 2012). Biofilm growth responds strongly to the addition of salmon carcasses, allowing marine-derived nutrients to persist in the environment, travel up the food web, and benefit rearing salmon in their natal streams (Albers, 2010). Many species of invertebrates, amphibians, and fish have physical adaptations allowing them to graze efficiently on biofilm (Kiffney and Richardson, 2001; Masclaux et al., 2012).

Another useful group of organisms for biomonitoring is aquatic invertebrates. This broad group includes all animals outside from the subphylum Vertebrata, but in practice describes mainly Arthropoda at the meso and macro scales, living in benthic or littoral areas of water bodies (Carter et al., 2006). Additionally, aquatic invertebrates can be classified by their feeding ecology based on identifiable physical features into functional feeding groups (Cummins and Klug, 1979), creating useful subdivisions for analysis. Of particular interest to Mt. Polley biomonitoring is the scraper feeding group, which is adapted to scrape and consume biofilm, forming a food web link with another biomonitoring target. Feeding groups can also be used to understand the flow of energy and chemicals through an aquatic food web.

Biofilms and invertebrates are appropriate for biomonitoring for a number of practical reasons: they are ubiquitous, found in abundance in most littoral areas, and reflect the environment from which they are sampled (Bonada et al., 2006; Costerton, 1995). They are easily collected, grown, stored and processed, without major ethical concerns nor substantial impacts on aquatic environments (Lewandowski and Beyenal, 2013). Their short life cycle means they reflect water conditions over days or weeks, rather than at one specific time, as would a water sample (Schindler, 1987). In biofilm, high microbial diversity, EPS secretion and multiple trophic levels allow for many potential sorptive pathways to be detected (Tien and Chen, 2013). Metal sorption into biofilms is dependent on a number of factors including species assemblage and successional stage (Comte et al., 2008), as well as water quality factors including temperature, pH, dissolved organic carbon, redox potential, and metal speciation (Chen et al., 2013).

1.6 METAL QUANTIFICATION TECHNIQUES

Several methods exist for quantifying metals present in solutions, and the proposed project will rely on two of these; one *ex situ* analytical technique and another *in situ* mass balance technique. The first of these is inductively coupled plasma mass spectrometry (ICP-MS), in which sampled materials (e.g. biofilm) are taken to a laboratory, digested in acid, then nebulized into an argon plasma torch with a temperature of the order of 10,000 K. This temperature is optimized by ionization energy so that metals and metalloids lose their most loosely bound electron. Ionized samples then pass by a magnet into a detector, determining the mass to charge ratio of the molecules, and reporting the concentrations of dozens of elements as accurately as parts per billion (Jenner et al., 1990).

An *in situ* method, which pairs well with biofilm monitoring, is that of diffusive gradients in thin-films (DGT). These devices detect bioavailable metals using a rate-controlled binding gel that simulates biotic ligands (Denney et al., 1999). This gel is packaged into a capsule with a circular window ~2 cm in diameter (Figure 1.4), and the device is left for several days *in situ*. The theory underpinning the use of DGTs is derived from Fick's Law, which describes diffusion across a membrane in the device. The concentration of metal in solution can be calculated based on the mass taken up by the device, in conjunction with diffusive rates that are specific to each metal as well as temperature and water quality factors (Guéguen and Dominik, 2003; Zhang and Davison, 1995).

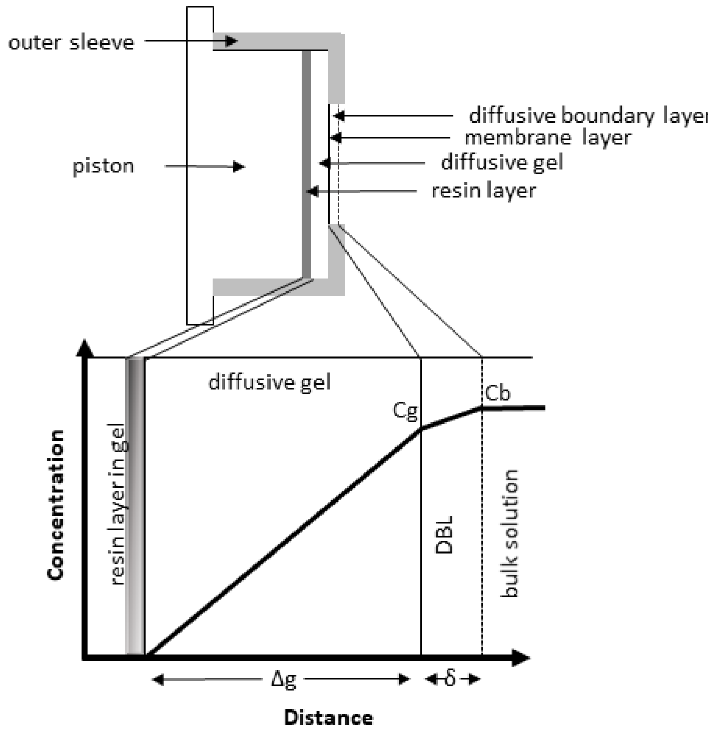

Figure 1.4 – A DGT piston sampler (top), with an idealized metal concentration gradient from the solution into the membrane (bottom). DBL=diffusive boundary layer, which is a region of metals diffusion extending into the water column. The variable Δg is the gel thickness, δ is the DBL thickness, Cg is concentration of metal at the gel interface, and Cb is concentration of metal in bulk solution. Piston and concentration charts adapted from Zhang and Davison (1995).

The DGT approach has several advantages over other methods used for determining metal concentrations. First, it only detects free metal ions that are able to pass through the membrane, unlike filtered water samples which detect all species of metals smaller than an operational filter size, often 0.45 µm (Guéguen and Dominik, 2003). Free ions which are detected by DGTs tend to be the most bioavailable forms of labile metals, a trait which lends this method particularly well to pairing with biomonitoring. In conjunction, biofilm and DGT monitoring will show both organismal and environmental concentrations of theoretically bioavailable trace metals. Like biofilm monitoring, DGT sampling is inherently time-integrated and reflects water conditions over days to weeks, unlike a water grab sample which reflects metals at a discrete point in space and time (Zhang and Davison, 2015). Finally, the DGT technique avoids substantial problems of sample contamination and metal re-speciation, which can otherwise occur at the collection and processing stages of *ex situ* methods (Denney et al., 1999).

1.7 The Free Ion Activity and Biotic Ligand Models

Bulk metal concentrations alone tend to be poor predictors of bioavailability in aquatic environments, because many species of metals are inert and unavailable to organisms (Paquin et al., 2002). As such, models incorporating more chemical factors such as redox potential, pH, and hardness have been developed, which predict the uptake of trace metals into organisms. The free ion activity model (FIAM) (Campbell, 1995) and the derived biotic ligand model (BLM) are useful for predicting the behavior and availability of metal ions in the environment (Di Toro et al., 2001). These models describe the behavior of dissolved metal cations, including their affinity to bind with ligands on organisms, specifically cell membranes for the FIAM, and gill surfaces for the BLM. Metal ions may also bind with dissolved organic matter and fine-grained sediment (e.g. silts and clays) and colloids (Chen et al., 2013). Under most environmental conditions other cations exist in solution, competing for the same binding sites as trace metal ions. Water hardness plays a major role in the bioavailability of trace metals, due to the tendency of Ca^{2+} to compete for binding sites with metals including Cu^{2+} (Di Toro et al., 2001).

Good biomonitoring practices are underpinned by theory (Bonada et al., 2006), so the goal of this research is not to perform modelling, but to utilize the FIAM framework to contextualize biomonitoring results. The FIAM describes that ionic metals in solution and ligands on organisms form surface metal complexes, and that the concentration of metal-ligand complexes is proportional to the concentration of metal ions in solution (Campbell, 1995).

Therefore, concentrations of trace metal in aquatic organisms should reflect the concentration of free metal ions in the water bodies where they were sampled. This is especially true for biofilm, as EPS is known to contain a high density of ligand sites (Comte et al., 2008), and biofilms do not take up metal through dietary exposure in the same manner as many aquatic invertebrates. This understanding allows for biofilm samples to be used to infer relative concentrations of free metal ions (i.e. bioavailable metals) in solution.

1.8 Trophic Transfers Indicated by Nitrogen Isotopes

Stable isotopes of nitrogen (N) can be used to better understand the transfer of nutrients across trophic levels. In the atmosphere, ^{14}N and ^{15}N occur in a predictable ratio of approximately 99.7% to 0.3%, respectively. When nitrogen enters the food web, the rarer ^{15}N isotope is enriched by 3-4 part per thousand in each trophic transfer, and the isotopic discrimination factor depends on the organism in question (Peterson and Fry, 1987). If the isotope ratio of a producer is known, this initial ratio will be modified by its consumers and increase at higher levels in the food web. Organisms at higher trophic levels in aquatic systems tend to concentrate ^{15}N, and which can be used to establish trophic positioning.

Analyzing the isotope ratios of N in biofilm will indicate the approximate trophic level of this multi-species assemblage, indicating its role in the ecosystem. Understanding food web linkages will support the analysis of metal transfer across trophic levels, as has been done by Jardine et al. (2013) in their study of mercury biomagnification in biofilm, invertebrates, and fish. The quantification of multiple isotope ratios is accomplished using continuous-flow isotope ratio mass spectrometry (IRMS), in which a small (~50 μg) sample is ionized and passed by a magnet into a series of detectors, one for each isotope, indicating their ratio in the sample (Spötl and Vennemann, 2003).

1.9 OBJECTIVES AND LIMITATIONS OF THIS RESEARCH

The primary goal of this thesis is to evaluate some of the spatial and trophic aquatic impacts caused by the Mt. Polley Mine tailings spill. This is to be done by exploring the biogeochemical impacts of the spill to samples of sediment and water, and low trophic level organisms in Quesnel Lake and nearby water bodies, including Polley Lake, and the Quesnel River. Biogeochemical analysis focuses on patterns in trace metal concentrations across spatial and trophic scales. Spatial analysis is accomplished by classifying sites based on whether they are within the pathway of spill materials, on their distance from the spill entry into Quesnel Lake at the Hazeltine Bay, and on their location on either the west or east sides of the Cariboo Island sill, a natural barrier between differently impacted areas of Quesnel Lake.

Trophic analysis employs two tools for the assessment of trophic positioning: organism classification into functional feeding groups, and isotope ratio chemistry. Collectively these measures allow for a broad evaluation of the spatial behavior and potential biotic harm from trace metals in impacted water bodies. Note that in this thesis, the term "trace metal" includes metals (e.g. Cu, Mn, V), metalloids (e.g. As), and one nonmetal (Se). This is a common operational classification used mainly for linguistic simplicity.

The overall objectives of this thesis (represented in Figure 1.5) are as follows:

1 Determine elemental patterns (i.e. metal ratios or metals found in conjunction) that identify material originating from Mt. Polley Mine tailings and discharge

2 Identify spatial patterns and concentration gradients of spill-associated metals in potentially impacted environments

3 Evaluate the relative influence of natural and anthropogenic inputs on metal concentrations in environmental samples from Quesnel Lake

4 Determine if biofilm may be a vector for spill-associated trace metals to enter the food web

5 Explore the behavior of trace metals at the base of the Quesnel Lake food web, including the relative importance of dietary and environmental exposure

6 Identify evidence for biotic accumulation and trophic transfer of spill-associated trace metals

The thesis is structured with an introduction chapter at the start (Chapter 1), with core chapters (2 – 3) following. Each core chapter is written as a stand-alone scholarly article, with individual introductions and conclusions. For this reason, certain explanatory topics are repeated. The first core chapter (Chapter 2) focuses on spatial analysis in environmental samples, with a focus on metals data from epilithic biofilms, and explores objectives 1, 2, and 3. The second (Chapter 3) analyses the base of the Quesnel Lake food web, including of abiotic and biotic samples, to assess the dietary and passive transfer of trace metal into biofilm and invertebrate feeding groups, seeking to address objectives 4, 5, and 6.

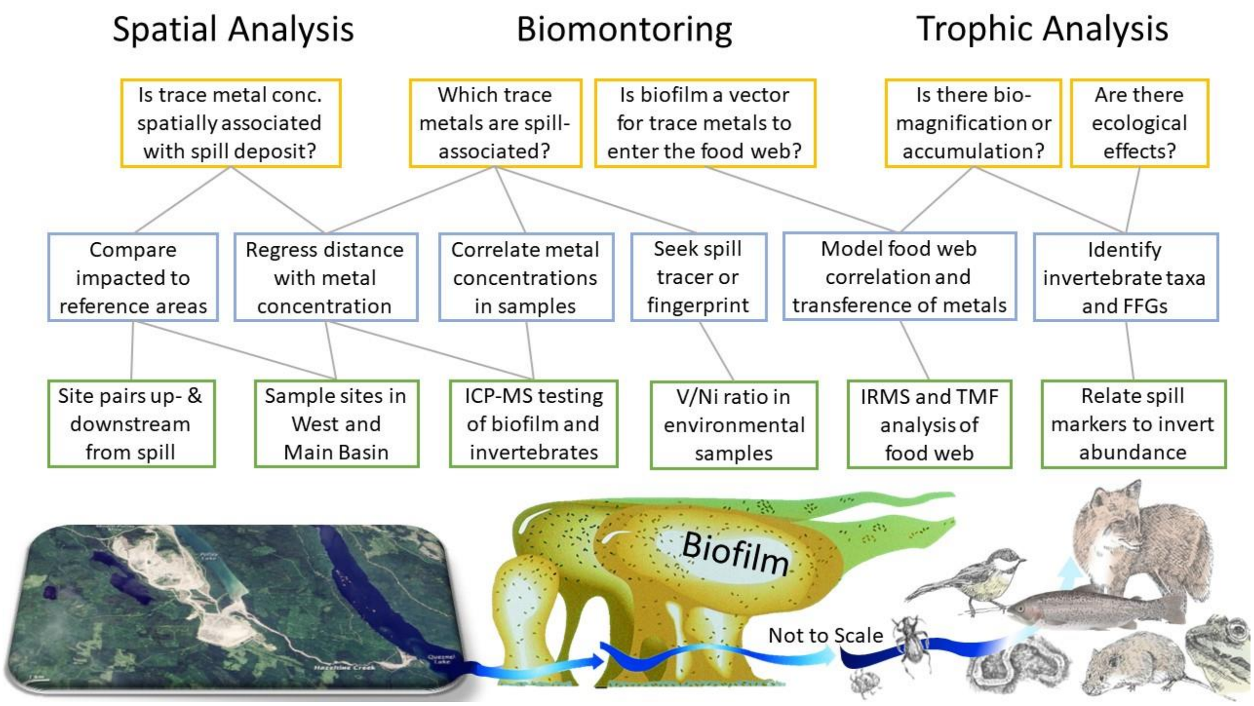

Figure 1.5 – A flow chart of questions, objectives, and methods in this research project, loosely categorized by their relationship to spatial analysis, biomonitoring, and trophic analysis. Questions are yellow, objectives are blue, and methods are green.

Two limitations of this study should be acknowledged. First, trace metals data in environmental samples are usually in the form of mass-based concentration in dried material (mg/kg dry wt.). Metals speciation, though influential, is not investigated due to its requirement for inordinate laboratory resources. Pursuit of speciation data would have precluded the possibility of exploring a broad suite of metals. The second limitation is the observational, rather than experimental, nature of this research, which makes it difficult to establish causality for observed effects. An ideal study design would acquire data from before and after a treatment, at both a control and impacted location. Because the subject of this study is the environmental impact caused by an unpredictable failure of a mine tailings storage facility, the “treatment” cannot be studied as such. Comparisons of differently impacted environments are made with the understanding that unimpacted (or lesser-impacted) environments are reference, rather than control groups. Despite these limitations, biomonitoring is shown to be an important and informative tool for studying the effects of trace metals in the Quesnel River Catchment.

2 Biomonitoring of Trace Metal Spatial Patterns

2.1 Introduction

In August 2014 a major mine tailings spill occurred in the Cariboo region of British Columbia, Canada. The tailings impoundment facility at the Mt. Polley Mine, a copper-gold open pit operation, was breached, releasing ~25 M m^3 of tailings, interstitial water, and construction material (MPMC, 2015c) into the landscape. Spill material partially filled nearby Polley Lake, and subsequently was redirected into the channel of nearby Hazeltine Creek causing a large amount of scoured surficial material to enter Quesnel Lake along with the tailings (Figure 2.1). Quesnel Lake is a long, deep fjord lake containing important habitat for anadromous salmon and other protected fisheries (Beacham et al., 2004). The majority of spill material settled to the bottom of Quesnel Lake in the West Basin, forming an extensive tailings deposit near the mouth of Hazeltine Creek. The large volume of inflowing material, estimated at 20 M m^3, caused Quesnel Lake to seiche, which spread the finer suspended materials into the Main Basin, and also downstream into the Quesnel River. These fine particles in suspension had a D_{50} ~1 μm and so remained in a turbidity plume for several months after the spill event (Petticrew et al., 2015). Although not acid generating, the total iron, copper, arsenic and manganese of the spilled tailings exceeded sediment quality guidelines for the protection of aquatic life (MPMC, 2015a). However, sequential acid extraction by the Tessier method showed those metals to be mainly in a “residual” form, indicating low levels of bioavailability (Sussbauer, 2017). Additionally, the Mt. Polley Mine resumed operations in 2015, and has continued to discharge its milling and processing wastewater directly into Quesnel Lake (Ministry of Environment, 2017). The impact of this spill and the continuing discharge are not fully understood, and monitoring the impact from the Mt. Polley spill is of concern to land managers and researchers alike.

Biomonitoring, the measurement of body burdens of potentially toxic chemicals in organisms, has been shown to be a useful tool for environmental monitoring, and has a number of advantages over chemical analysis of water or sediment, many of which are applicable to the situation at Mt. Polley. Biomonitoring provides direct information about the health of ecosystem constituents (Zhou et al., 2008), which should help delineate the impact of the spill in Quesnel Lake, given the lake's unusual water movement and limnology (Laval et al., 2008), and the biogeochemical nature of the spill. The risk posed by spilled materials could be substantiated by testing organisms for their metal content, which has been done in other mine-impacted landscapes (Arini et al., 2012; Farag et al., 1998).

Many organisms and strategies can be used for biomonitoring, but biofilms are particularly suitable. They are easily grown and sampled, are responsible for the majority of littoral primary production (Hobson et al., 1995), and contain many biotic ligand sites where metals can be adsorbed (Hao et al., 2013). These ligand sites are important because they establish a theoretical link between biofilm metals sorption and bioavailable metals in solution, as modelled by the free ion activity model (FIAM). This model describes the behavior of cationic trace metals in the water column, as they relate to organisms and uptake (Campbell, 1995). Biofilm sampling for trace metals can serve a dual purpose of monitoring metal contributions from the Mt. Polley Mine spill into the environment, and assessing the impact of those metals at the base of the food web.

Another group of organisms in the lower portion of the aquatic food web is invertebrates, which are also well suited to biomonitoring of the Mt. Polley spill. Invertebrates fulfill many ecological roles near the bottom of the aquatic food web, and are useful to biomonitoring because of our ability to subdivide them into meaningful groups that provide additional information about trophic and food web transfers (Farag et al., 1998). This additional layer of information indicates the extent of trace metals transference through the food web, and the potential fate of trace metals that do enter the food web.

Aquatic invertebrates in this study are classified into one of four well established functional feeding groups which are defined by organismal morphology, as first developed by Cummins and Klug (1979). The feeding groups utilized by this study are: scrapers, which feed by scraping and consuming epilithic biofilm from their environment; filterers, which have specialized appendages or create silk nets to capture suspended or dissolved organic matter; predators, which feed on other heterotrophs; and collectors, a generalist group which gathers organic matter from places where it is accumulated by fluvial processes. This chapter focuses on collectors and predators, which were sampled in abundance and represent the secondary and tertiary trophic positions.

Trace metal impacts usually take time to equilibrate in aquatic systems (Morin et al., 2008) and signs of contamination are typically found at low trophic levels (Timmermans et al., 1989), particularly among organisms living at the sediment–water interface (Santschi et al., 1990). Biofilms, which are microbial assemblages living in excreted extracellular polymeric substances (EPS) are an appropriate monitoring choice in many aquatic systems due to their ubiquity and ease of sampling (Tien and Chen, 2013), and for the chemically active nature of EPS, which binds metals in accordance with the FIAM (Sheng et al., 2010; Worms et al., 2006). While it is difficult to predict ecosystem impacts from the trace metal content of water and sediment, organismal concentrations are a better indication of ecosystem damage as they indicate the consequences of an impact, rather than quantifying the impact itself (Ravera, 2001).

To assess the suitability of biomonitoring, Bonada et al. (2006) have identified 12 criteria for a theoretically ideal biomonitoring program (Table 2.1). Although the criteria are written in the context of invertebrate biomonitoring, they are widely applicable. With the goal of exploring the chemical impacts of the Mt. Polley tailings breach, biofilm and invertebrate biomonitoring for trace metals in tissues fulfil the majority of these criteria.

Table 2.1 – A qualitative assessment of biofilm and invertebrate trace metals monitoring, according to their level of fulfilment (high, moderate, or low) of criteria for an ideal biomonitoring program described by Bonada et al. (2006)

Ideal Biomonitoring Criteria (Bonada et al., 2006) Category Criterion	**Level of Fulfilment by Biofilm Monitoring**	**Level of Fulfilment by Invertebrate Monitoring**
Rationale		
(I) Derived from sound theoretical concepts in ecology	High	High
(II) A priori predictive	High	High
(III) Potential to assess ecological functions	Moderate	Moderate
(IV) Potential to discriminate overall human impact (i.e., to identify anthropogenic disturbance)	High	Moderate
(V) Potential to discriminate different types of human impact (i.e., to identify specific types of anthropogenic disturbance)	Low	Low
Implementation		
(VI) Low costs for sampling and sorting (field approaches) or for standardized experimentation (laboratory approaches)	High	High
(VII) Simple sampling protocol	Moderate	High
(VIII) Low cost for taxa identifications (no specialists in taxonomy required)	Low	Moderate
Performance		
(IX) Large-scale applicability (across ecoregions or biogeographic provinces)	High	High
(X) Reliable indication of changes in overall human impact	High	High
(XI) Reliable indication of changes in different types of human impact	Low	Low
(XII) Human impact indication on linear scale	Moderate	Moderate

Biomonitoring of metal concentrations in communities of biofilm fulfills nine, while invertebrate monitoring fulfils 10, of the 12 Bonada et al. (2006) criteria to some degree. In this context, biofilm and invertebrate monitoring are both deficient in the ability to distinguish between distinct types of anthropogenic trace metals impacts (criteria V and XI), and biofilm is not able to be easily taxonomically identified (criterion VIII). These deficiencies are important, because it would be useful in Quesnel Lake to differentiate distinct impacts from the Mt. Polley tailings spill of 2014 and the ongoing wastewater discharge starting in 2015. Despite this, biomonitoring may help address the effects of trace metals in the Quesnel Lake region, especially relating to biotic uptake.

It is hypothesized that trace metal impacts from the Mt. Polley spill will manifest themselves spatially in biologic materials, with higher concentrations of metal in materials sampled in areas closer to the spill deposit. To test this hypothesis, trace metal concentrations in sediment, the bodies of invertebrates, and collected biofilms of potentially impacted water bodies are regressed with distance to the site of the spill. Additionally, biofilm metal concentrations in the West Basin of Quesnel Lake, which contains the majority of spill impacts, are compared with those in the lesser-impacted Main Basin in a binary fashion. The null hypothesis is that proximity to the Mt. Polley spill deposit has no relation to the concentration of trace metals in environmental samples from the Quesnel Catchment.

2.2 METHODS

2.2.1 Field Procedures

To represent potentially spill-impacted water bodies, seven sites were chosen to represent Quesnel Lake, the Quesnel River, and Polley Lake. All these areas could have received particulate and dissolved metals from the Mt. Polley Spill, but to differing degrees. Each of the seven potentially impacted sites is paired with another nearby site, which is upstream of impacted water bodies, and therefore unimpacted by the spill. Each Quesnel Lake site is paired with a nearby tributary stream flowing directly into the lake. Polley Lake is paired with the upstream Frypan Lake, and the Quesnel River is paired with the Cariboo River (Figure 2.1). This results in 14 principal sites which all received the same sampling procedures. Sampling took place during two distinct 42-day periods in 2016: in the spring (April 13 to May 26), and in the summer (June 2 to July 14). Spring sampling was intended to capture conditions during lake overturn and spring freshet, and summer sampling to represent conditions of low stream flow and lake stratification. Procedures were the same for both sampling periods.

Figure 2.1 – Sampling site map of tile-grown biofilms in Quesnel Lake and surrounding water bodies. Sites labelled as “pair” indicate a site in a stream that flows into Quesnel Lake, and another site in the littoral zone adjacent to the stream, in Quesnel Lake itself.

Biofilm was grown on artificial substrates and sampled on the 42nd day of both sampling periods. Unglazed clay tiles were used, each 15 x 15 cm, and 12 tiles were placed at each site on day 0, totaling 2700 cm^2 of artificial substrate. Tiles were custom manufactured to avoid metal contamination, and made out of Plainsman M340, a white clay free from industrial refinement and chosen for its low metal content (Hansen, 2016). At Quesnel Lake sites, tiles were hung on moorings in the photic zone between 0.3 to 3.6 m depth, with 30 cm spacing between tiles. In stream sites and in Polley and Frypan Lakes, tiles were anchored to cement blocks and left in water of 30 - 100 cm depth in stream pools and protected near-shore environments with minimal overhead cover.

Biofilm collection consisted of manual removal of tile-grown biofilm using acid-washed plastic scraping tools, and storage in sterile 50 ml conical tubes in refrigerated containers. In addition to artificial substrates (tiles), natural substrates (rocks) were also sampled for biofilm on the 42nd day of both sampling periods. Natural substrate samples were taken from the same bay, riffle, or pool and within 20 m from the artificial substrate tiles. These samples were chosen based on the smoothness of the stone, and abundance of biofilm. Smooth sides of cobble-sized stones were scraped completely, and their surface area was measured by covering and tracing them using transparent plastic film. All biofilm samples were frozen the same day of sampling, after returning from the field.

Overlapping with the 42-day growth period of biofilm, invertebrates were sampled from the same sites, five times on a weekly schedule, on the 7th, 14th, 21st, 28th, and 35th days of the sampling period. A kick net sampler with 800 µm mesh size and a 460 x 230 mm frame was used for invertebrate capture, followed by field elutriation in 20 L acid-washed buckets. At each site, substrate was perturbed by kicking/resuspending while walking backwards at a rate of ~0.5 m/s, repeated three times for one minute each. Samples were refrigerated following field collection, and invertebrates were separated from debris and frozen within 24 hours of capture.

Five invertebrate samples from each site and season were combined to obtain sufficient volumes for analysis.

Weekly water quality measurements were also taken at each site, on days 0 (when tiles were placed), 7, 14, 21, 28, 35, and 42. Water quality data consisted of temperature and pH measurements (Myron L Ultrapen PT2), and turbidity (Analite NEP 9000 portable probe). In some circumstances these instruments were supplemented with a Eureka Manta2 multi-sonde, which records pH, temperature and turbidity. All instruments were calibrated with the same turbidity and pH standards at intervals recommended by the manufacturer.

In addition to the purpose-grown biofilm sampled from artificial tiles in the spring and summer, 14 additional biofilm samples were recovered from plastic buoys in the fall, on November 27 - 28, 2016. These samples were not grown under the same controlled conditions as tile-grown biofilms, but were recovered from submerged spherical buoys at 2 - 20 m depth that were part of another monitoring program by the British Columbia provincial government (L. Williston, pers. comm., 14 Jun 2016). Buoy-grown biofilm grew for approximately six months, from April - November 2016, overlapping spatially and temporally with the tile-grown biofilm. Utilizing these additional samples expands the geographical scope of the project considerably, adding sites in the otherwise unsampled East Arm (Figure 2.2). Biofilms from buoys were sampled in a similar manner as other samples, using acid-washed plastic scraping tools, and by scraping a 10 x 10 cm area thoroughly into sterile 15 ml conical tubes. Comparisons between buoy samples and other principal artificial substrates are imperfect, because buoy and tile biofilms were grown under different conditions, and buoy biofilms do not have paired sites from inflowing water bodies. For these reasons, comparison of biofilm data from different sources should be interpreted with caution. Clean techniques per Benoit et al. (1997) were practiced during field sampling and laboratory work, including wearing gloves, using disposable or acid-washed instruments, and minimizing contact with samples.

Figure 2.2 – All biofilm sampling sites in the Quesnel Lake area. Tile sites are indicated by yellow squares, where biofilm was purpose-grown and sampled in the spring and summer 2016. Buoy sites are indicated by red circles, where biofilm was recovered from submerged buoys in November 2016. Note that tile sites are clustered towards the west near the Mt. Polley Mine, while buoy sites are spread throughout the West and East Arms of the lake.

2.2.2 Biofilm Laboratory Work

Following the summer sampling period, biofilm samples were weighed whole, then homogenized using acid-washed stainless-steel dissection tools, and split into four subsamples to undergo ashfree dry mass determination, chlorophyll a analysis, metals testing by inductively coupled plasma mass spectrometry (ICP-MS), and stable isotope analysis. Ash-free dry mass determination was performed following Steinman et al. (2007). Small (< 0.5 g) samples were first dried in a 70° C oven and weighed dry, then ashed for 1 h at 500° C and weighed again to determine the ash free dry mass, as well as inorganic and organic proportional content.

To dry and preserve samples, the remaining biofilm fraction was lyophilized using a Labconco Freezone 6 drier, in conditions below -50° and under 5 Pa. To determine chlorophyll content, subsamples containing 0.01 g of dry biomass (calculated using ash-free dry mass data) were separated for chlorophyll analysis. Following EPA Method 446 with slight modifications, samples were steeped using 10 ml of 90% acetone for 18 hours, then the elutriate was transferred to cuvettes so the absorbance could be measured at 750, 664, 647 and 630 nm (Arar, 1997). To calculate chlorophyll a and correct for phaeopigments following the equations of Lorenzen (1967), the cuvette was then acidified with 0.06 ml of 0.1 N hydrochloric acid, and its absorbance was measured at 665 and 750 nm. Precautions were taken at all previous steps to minimize the light exposure received by biofilm samples, including storage in dark containers, minimize handling, and work in dimly lit environments. Remaining biofilm biomass was set aside for elemental analysis.

Additional subsamples (~50 µg) of freeze-dried biofilm were taken from remaining material, and packed into tin capsules for isotope ratio mass spectrometry (IRMS). This analysis was done off-site by the University of California - Davis Stable Isotopes Facility, using a continuous-flow isotope ratio mass spectrometry. Specifically, a PDZ Europa ANCA-GSL elemental analyzer was used, interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Data were returned in the form of enrichment of two isotopes: carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$), relative to known standards.

Homogenized subsamples of biofilm were used for acid digestion and elemental analysis. Samples of 0.5 g were separated and placed into acid-washed propylene 15 ml tubes with a mixture of 3.5 mL nitric acid and 1.5 mL hydrochloric acid, then heated via a block digester to 90° C, and held at that temperature for seven hours before cooling, representing a modified version of EPA Method 3051A (Link et al., 1998). For matrix matching, 10 ml of reagent-grade distilled water was then added. A technician ran each sample through an ICP-MS unit (Agilent Technologies 7500cx Series with MicroMist nebulizer). Data were returned in µg/g for 32 metals, nonmetals, and metalloids, including copper, vanadium, aluminum, selenium, iron, lead, mercury, and manganese. Each batch of 12 samples for metal analysis included a reagent blank, a reference material (NIST SRM 1573a - derived from tomato leaves), and a calibration standard containing 10 mg/L of all tested metals. Each batch of 12 samples also contained a duplicate of an existing sample, and another duplicate that was spiked with 1 ml of the 10 mg/L standard solution. The ICP-MS was calibrated every third batch of 12 samples.

2.2.3 Invertebrate Laboratory Work

Invertebrate samples, frozen following field work, were later thawed and sorted under a dissecting microscope. The original samples of invertebrates (collected weekly) from each site and sampling period were separated into four functional feeding groups and stored in fresh tubes, representing all invertebrates of the feeding group, per site, per sampling period. The four feeding groups used were: scraper, collector, predator and filterer, and classification was performed following a key by Merritt and Cummins (1996). Generalist feeders such as many Amphipoda and Plecoptera were designated as collectors.

In addition to the feeding groups, 13 common taxonomic groups, listed in Table 2.2, were counted using a key from Hauer and Resh (2007). Taxonomic groups were used for counts of relative abundance by date of sample collection, but invertebrates were not separated into subsamples based on taxonomy. Most taxonomic groups do not correspond with a specific functional feeding group. Oligochaeta, which are small (<1 mm) and difficult to identify based on morphology (Seesao et al., 2017), were identified only to phylum. Most other invertebrates were identified to order, and the order Diptera was subdivided into three families.

Table 2.2 – Taxa used for invertebrate classification, following a dichotomous key from Hauer and Resh (2007), and the functional feeding groups (FFGs) represented by each taxa. Classification end-points are bolded, with common names in parentheses.

Phylum	Class	Order	Family	FFGs
Oligochaeta (earthworm)				C
Crustacea	Malacostraca	**Amphipoda** (scuds)		C, P, F
Arthropoda	Arachnida	**Hydrachnidia*** (water mites)		P
Arthropoda	Insecta	**Trichoptera** (caddisflies)		F, C, P,
Arthropoda	Insecta	**Coleoptera** (beetles)		P, C
Arthropoda	Insecta	**Plecoptera** (stoneflies)		C, S, P
Arthropoda	Insecta	**Ephemeroptera** (mayflies)		S, C, P
Arthropoda	Insecta	**Odonata** (dragonflies)		P
Arthropoda	Insecta	**Hemiptera** (true bugs)		C, P
Arthropoda	Insecta	**Diptera** (true flies)	Not the following:	C, P, S
Arthropoda	Insecta	Diptera	**Chironomidae** (midges)	C
Arthropoda	Insecta	Diptera	**Simuliidae** (black flies)	F

Note: The taxon *hydrachnidia* is an unranked classification, not an order, so is marked with an asterisk (*). The feeding groups represented by each taxa are listed in order of highest to lowest abundance, and abbreviated to the first letter (**C**ollector, **S**craper, **P**redator, **F**ilterer).

Following invertebrate identification, samples of invertebrates (separated by functional feeding groups) were lyophilized, then homogenized by sterile ball grinding. These samples were processed for ash free dry mass, trace metals by ICP-MS, and stable isotope ratios by IRMS. The procedures for these three analyses, described in section 2.2.2, were identical for invertebrate and biofilm samples.

2.2.4 Statistics and Data

Artificial substrate biofilm results, which had lower variance of metal concentrations but similar means when compared to natural substrates (see Section 3.4), are used in all statistical analyses, except for two sites in the summer sampling period where artificial substrate biofilm was unavailable: Winkley Creek and Quesnel Lake at Abbott Creek. Summer data for these sites were substituted by using natural substrate biofilm (gathered at the same place and time), for statistical tests and in diagrams.

Sediment data for Quesnel Lake are obtained from another study (E. Petticrew, unpublished data, 2016) in which core samples were taken from many benthic areas of Quesnel Lake in 2016, and analyzed for metal content, particle sizes composition, and other sediment properties. At sites in the West Basin of Quesnel Lake, nearby cores are available at all biofilm/invertebrate sample sites. In those areas, sediment cores were collected from within 1 km of organism samples. Distances to coring sites in the Main Basin are larger, due to limited sampling in that region. These distances are ~4.6 km between the Abbott site and its sediment core, and ~2.5 km between the Whiffle site and its sediment core. The distinct locations of sediment cores are reflected in the distance values in Table 2.3.

Sediment samples in this study are not intended to represent the substrate in which organisms were living, but to represent landscape-scale variations in sediment characteristics and reflect the broader difference between West Basin (tailings-impacted) and Main Basin (relatively unimpacted) sediments. Metal concentrations from the top 1 cm of sediment in each core were used to represent sediment at the corresponding site. Polley Lake sediment data were based on a published post-spill dredge sample from summer 2015 (MPMC, 2016). Quesnel River sediment data were taken from Sussbauer (2017), who calculated the mean metals content of 64 samples of resuspended channel bed sedimentation taken between fall 2014 and fall 2015. Due to the variety of data sources, not all metals in sediment are available at all sites, and the results should be interpreted cautiously.

The sites in Quesnel Lake at Abbott and Whiffle Creeks are located in the relatively unimpacted Main Basin, and are treated as reference sites to determine whether the West Basin of Quesnel Lake varies from the Main Basin in trace metal concentrations. The Main Basin did receive measurable spill impacts in the form of suspended materials spread up-lake by seiches in the aftermath of the spill, but to a much lesser degree than the West Basin (Petticrew et al., 2015). Linear regression is used to establish a relationship between distance from the spill site and metal concentration in different environmental materials. Data were checked for normality using skewness and kurtosis testing. In Section 3.3.1, t-test results are presented, and in some cases these results are based on small sample sizes ($n = 14$), which strains parametric assumptions. These results can still be used to visualize differences among means and variances in samples and help to identify instances where elemental concentrations differ greatly in magnitude. Statistics were done using Stata 13.0 (StataCorp, 2013), with $\alpha = 0.05$ for all tests.

Preliminary assessments of parametric assumptions were done for all tests using the skewness/kurtosis test for normality. The West and Main Basins of Quesnel Lake were compared in the spring and summer using unpaired t-testing, when parametric assumptions were met. Pairwise correlations were performed using Pearson's product-moment correlation coefficient. Least-squares linear regression was used for statistical testing on distance models.

Distance models were used to relate analyte concentration and distance from the mouth of Hazeltine Creek. In these models, Polley Lake is given a distance of zero. In Quesnel Lake, sites up the hydraulic gradient from Hazeltine Creek (i.e. east) were designated negative distance values and those down the hydraulic gradient (i.e. west) were designated positive distance. Distances were measured using polygonal paths in Google Earth, following the approximate thalweg of flow in Quesnel Lake from the mouth of Hazeltine Creek to the location of biofilm sampling (Appendix A). Spring and summer sampling periods were treated as repeat measures. Stata 13.0 was used for statistical analysis (StataCorp, 2013), with α of 0.05.

2.3 Results

2.3.1 Patterns in Biofilm Trace Metal by Season, Basin, and Substrate

If tile-grown biofilm trace metal concentrations are compared between the differently impacted West and Main Basins of Quesnel Lake, a trend emerges that spill-associated metals (Appendix B) tend to be higher in the West Basin, and also during the spring sampling period (Figure 2.3). In this analysis, the Quesnel River site (labelled on Figure 2.1 as QRRC, is ~1 km downstream from the outlet of Quesnel Lake) is treated as part of the West Basin, which is consistent with a design comparing relatively impacted areas to reference areas. Only two sites, each sampled in two seasons, are available in the Main Basin, the midpoint of which is presented here with no standard deviation.

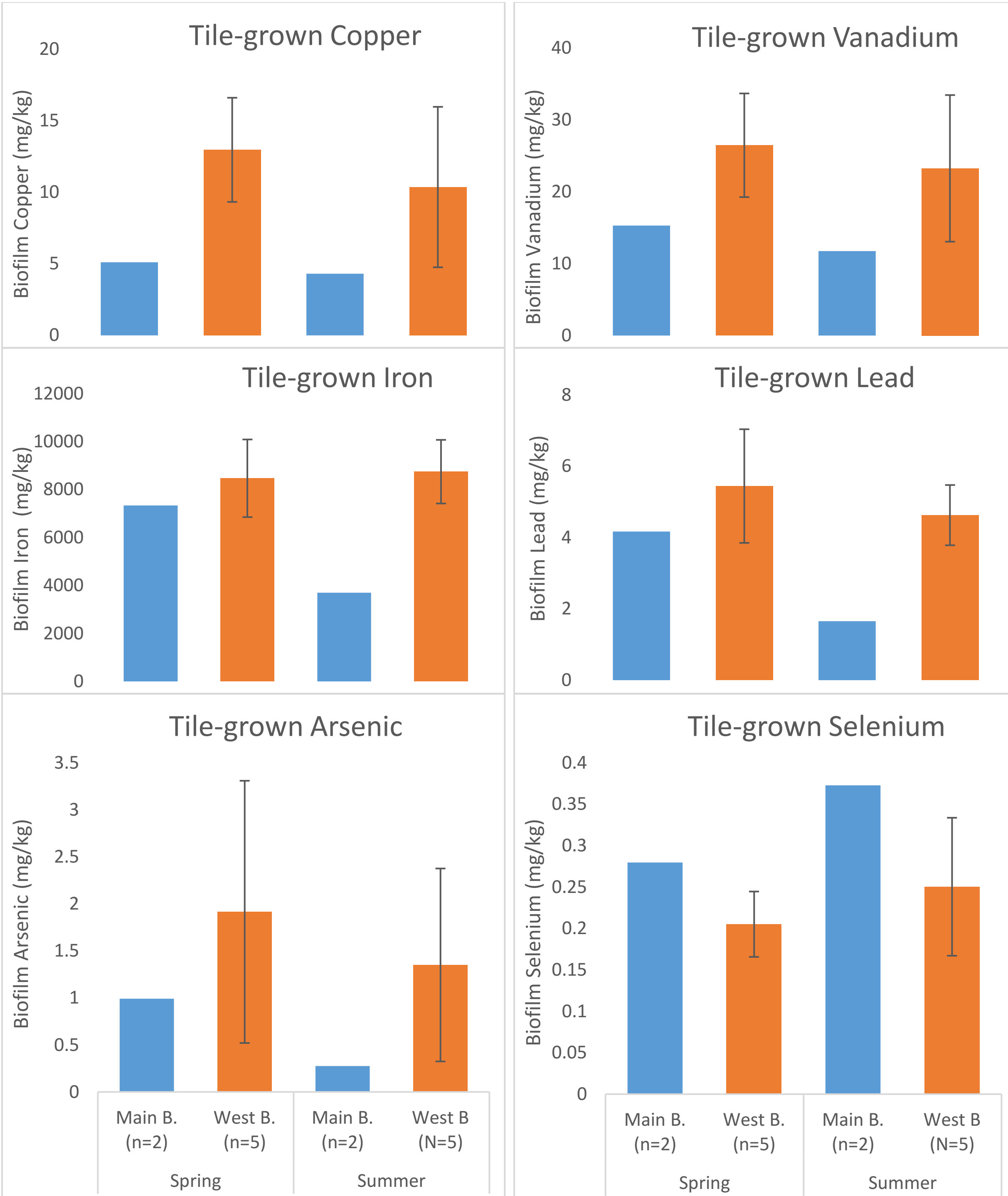

Figure 2.3 – Means or midpoints of metal contents, with standard deviations (calculated using the n-1 method) when appropriate, of tile-grown biofilms from Quesnel Lake and the Quesnel River (treated as West Basin). Data are separated by season and basin.

Sample sizes are too small for parametric testing when separated by season (n = 7), but trends in the data are apparent that are still useful for interpretation. The West Basin has higher mean concentrations of copper, vanadium, lead, and arsenic when compared to the Main Basin, by approximately double. The concentrations of these metals in biofilm is also highest during the spring sampling period. The opposite seasonal and spatial patterns are observed in biofilm concentrations of selenium, which tends to be highest in the Main Basin, and during the summer sampling period.

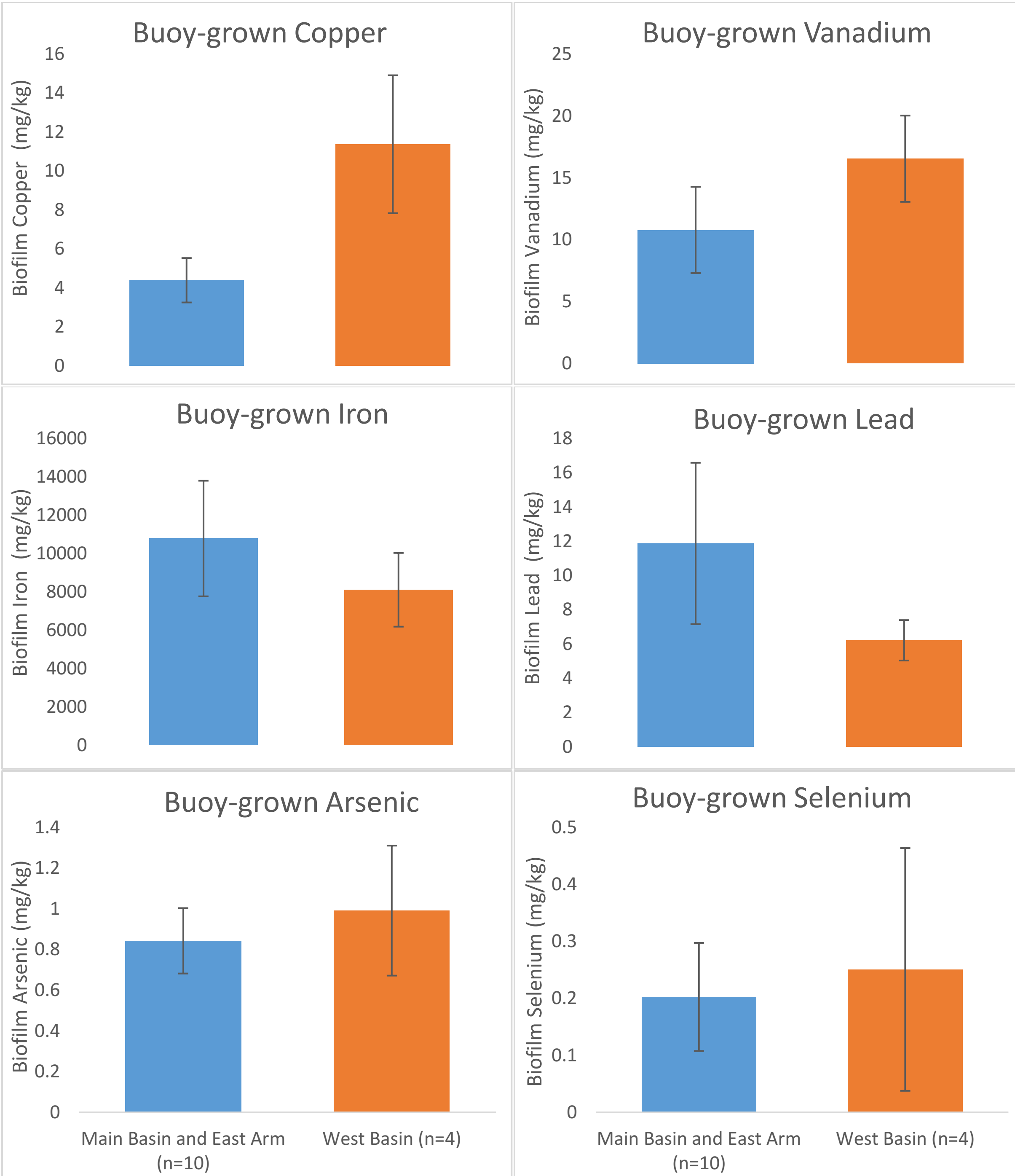

Figure 2.4 – Mean metal concentrations and standard deviations (calculated using the n-1 method) in buoy-grown biofilms, separated by basin (i.e. east or west of the Cariboo Island Sill) in Quesnel Lake.

Buoy-grown biofilms from the West Basin are significantly higher in their concentrations of copper ($t = 3.864$, $p = 0.013$, $n = 14$) and vanadium ($t = 2.130$, $p = 0.048$, $n = 14$) when compared to other areas of the lake. Arsenic and selenium also have higher mean concentrations in the West Basin, though not significantly different. The opposite pattern is expressed by lead ($t = 3.530$, $p = 0.002$, $n = 14$) and iron ($t = 1.97$, $p = 0.04$, $n = 14$), which have significantly higher mean concentrations in the Main Basin and East Arm.

2.3.2 Distance and Metal Content of Environmental Samples

The following tables (Tables 2.3 - 2.6) show metal concentrations at each of the seven Quesnel Lake sites for biofilm, invertebrates, and nearby sediment samples. The collector and predator functional feeding groups are presented because they represent the second and third trophic positions, and these feeding groups have the most samples available.

2.3.2.1 Sediment Metal Concentrations

Sediments sampled from near biomonitoring activities (Table 2.3) frequently exceed probable effects levels (PEL) from the Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines for the protection of aquatic life in the West Basin for copper (PEL: 197 mg/kg) and arsenic (PEL: 17 mg/kg) (CCME, 2018). While no aquatic sediment guideline exists for selenium, many Quesnel Lake sediment samples exceed the CCME *soil* quality guideline for environmental health (1 mg/kg). All sampled sites are near or above this threshold, but Polley Lake sediments have an anomalously high selenium content of 4.43 mg/kg, which is two to four times higher than all other sites.

Table 2.3 – Metal concentrations and distances to the sampling location of sediments in the Quesnel Lake area, using data from multiple sources. Polley Lake and Quesnel River sites are located in those water bodies. Other sites are in Quesnel Lake, and named after nearby tributary streams. Sediment sampling locations correspond with nearby biofilm and invertebrate collection sites, but are not identical sites, which is reflected in differences between distance variables.

	Dist. from		Metal Concentration in Sediment (mg/kg dry wt.)						
Site	Spill (km)	n	**Cu ~**	Fe	As	Se	**Pb ~**	Mn	**V ~**
Abbott (ref)	-19.10§	1	77.2	61200	16.9	0.95	26.4	6720	74.0
Whiffle (ref)	-15.13§	1	102	47900	13.5	1.53†	16.5	2600	80.1
Winkley	-4.71§	1	283†	46000	16.2	1.56†	16.3	2340	88.4
Polley	0.00	1	823†	29800	14.0	4.43†	9.3	2574	115
Raft	0.68§	1	796†	29100	14.0	0.97	12.7	1130	96.5
Cedar	8.69§	1	154	46600	93.2†	1.22†	12.9	3820	103
Quesnel R.	15.70	64	207†	39700	35.1†	2.30†	NA	1217	89.0

Note: Sites in the Main Basin of Quesnel Lake are treated as reference sites. A tilde (~) next to a bolded header indicates a significant correlation between absolute distance from the spill and sediment metal content. A section sign (§) next to a distance indicates this measurement differs from distances to biofilm and invertebrate sampling locations. A dagger (†) indicates the sample exceeds probable effect levels from CCME sediment quality guidelines for copper or arsenic, or CCME soil quality guidelines for selenium.

Using least-squares linear regressions, the data show a significant relationship between absolute distance from the spill and sediment-associated copper (R^2 = 0.692, p = 0.020), lead (R^2 = 0.818, p = 0.013) and vanadium (R^2 = 0.702, p = 0.018). Of these metals, copper and vanadium are frequently higher in sediments at West Basin sites compared to references. Contrarily, the regression with lead is negative, meaning it is relatively low at sites near the spill inflow when compared to more distant sites.

2.3.2.2 Biofilm Metal Concentrations

Biofilm metal values (Table 2.4), show significant spatial impacts for copper, arsenic, lead, and vanadium, which may be related to the Mt. Polley spill. Biofilm metal concentrations indicate a general pattern of higher values in the vicinity of the spill deposit as well as in the downstream direction of Quesnel Lake.

Table 2.4 – Metal concentrations in tile-grown biofilms in potentially spill-impacted areas. Polley Lake and Quesnel River sites are located in those water bodies. Other sites are in Quesnel Lake, and named after nearby tributary streams.

	Dist. from		Metal Concentration in Biofilm (mg/kg dry wt.)						
Site	spill (km)	n	**Cu ~**	Fe	**As ~**	Se	**Pb ~**	Mn	**V ~**
Abbott (ref)	-26.30	1	3.76	6632	0.88	0.24	3.49	220	12.7
Whiffle (ref)	-17.53	2	4.19	7476	0.70	0.23	4.08	548	14.4
Winkley	-5.86	2	5.33	8548	0.73	0.21	5.39	320	17.6
Polley L.	0.00	2	16.9*	9267	0.94	0.31	5.37*	215	33.5*
Raft	0.98	2	12.3*	7931	0.93	0.20	3.46	369	22.8*
Cedar	8.96	2	11.2*	7302	2.00*	0.26	5.18*	412	17.3
Quesnel R.	15.70	2	12.4*	9984	3.56*	0.16	5.78*	175	33.0*

Note: The relatively unimpacted Abbott and Whiffle sites in the Main Basin of Quesnel Lake are treated as reference sites. An asterisk (*) next to a bolded header indicates significant difference between the impacted (n = 2) and reference (n = 3) sites. A tilde (~) next to a bolded header indicates a significant correlation between distance and biofilm metal concentration.

Least-squares regressions, accounting for repeat measures, shows statistical significance between distance from the spill and biofilm copper ($R^2 = 0.474$, $p = 0.001$, $n = 13$), vanadium ($R^2 = 0.429$, $p = 0.014$, $n = 13$), and lead ($R^2 = 0.224$, $p = 0.002$, $n = 13$). Arsenic requires log transformation to meet normality assumptions, following which it is also found to be significant by this analysis ($R^2 = 0.534$, $p = 0.025$, $n = 13$). Iron, selenium and manganese do not follow this same pattern. Selenium is highest in Polley Lake biofilm, but this difference is not significant compared to reference sites. Biofilm manganese and iron concentrations have very little spatial relation with areas impacted by the Mt. Polley Mine.

2.3.2.3 Collector Invertebrates

Aquatic invertebrates from the collector feeding group tend to be higher in trace metals at areas downstream from the spill (Table 2.5). A significant regression is found between distance from the spill and collectors' concentrations of copper ($R^2 = 0.271$, $p = 0.023$, $n = 13$). Vanadium and lead concentrations in collectors also tend to be high toward the west side of Quesnel Lake, though this relationship is not found to regress significantly.

Table 2.5 – Metal concentrations in collector invertebrates from potentially spill-impacted areas. Polley Lake and Quesnel River sites are located in those water bodies. Other sites are in Quesnel Lake, and named after nearby tributary streams.

Site	Dist. from spill (km)	n	Metal Concentration in Collector Invertebrates (mg/kg dry wt.) **Cu ~**	Fe	As	Se	Pb	Mn	V
Abbott (ref)	-26.30	2	29.26	1647	0.88	2.27	0.34	94.7	1.85
Whiffle (ref)	-17.53	2	28.01	1512	1.67	2.02	0.39	175	3.88
Winkley	-5.86	2	25.81	1470	1.79	1.51	0.39	86.9	3.12
Polley L.	0.00	2	31.80	808.9	2.78*	3.85*	0.78*	177	2.41
Raft	0.98	2	38.94	2049	1.59	1.38	0.33	79.3	6.38*
Cedar	8.96	2	34.53	1876	3.90*	1.91	0.49	229	4.72
Quesnel R.	15.70	2	36.38	1938	1.90	1.89	0.55	119	4.34

Note: The relatively unimpacted Abbott and Whiffle sites in the Main Basin of Quesnel Lake are treated as reference sites. An asterisk (*) indicates significant difference between the impacted (n = 2) and reference (n = 4) sites. A tilde (~) next to a metal header indicates a significant correlation between distance and collector metal concentration.

Significant differences from the reference sites are found in Polley Lake collectors for arsenic, selenium, and lead, indicating there is some relationship between location and invertebrate uptake of these metals. However, significant differences between individual West Basin and reference sites are only found for collector arsenic (at Cedar Creek) and vanadium (at Raft Creek).

2.3.2.4 Predator Invertebrates

Invertebrate predator metals follow a similar pattern to other environmental samples and appear to be elevated in association with Mt. Polley Mine spill material (Table 2.6). Significant regressions are found between distance and log-transformed predator copper ($R^2 = 0.299$, $p = 0.024$, $n = 14$) and log-transformed vanadium concentrations ($R^2 = 0.724$, $p = 0.003$, $n = 14$), as well as between absolute distance and predator lead concentrations ($R^2 = 0.542$, $p = 0.009$, $n = 14$). High correlation coefficients are found, particularly for vanadium, and the pattern is supported by many West Basin predator samples that are significantly different in copper, lead, and vanadium compared to references.

Table 2.6 – Metal concentrations in predator invertebrates from potentially spill-impacted areas. Polley Lake and Quesnel River sites are located in those water bodies. Other sites are in Quesnel Lake, and named after nearby tributary streams.

Site	Dist. from spill (km)	n	Metal Concentration in Predator Invertebrates (mg/kg dry wt.) **Cu ~**	Fe	As	Se	**Pb ~**	Mn	**V ~**
Abbott (ref)	-26.30	2	32.26	409.0	1.07	3.28	0.07	78.24	0.24
Whiffle (ref)	-17.53	2	30.84	1005	0.52	2.92	0.11	56.76	0.68
Winkley	-5.86	2	40.68*	995.7	0.73	3.31	0.27*	78.78	1.59
Polley L.	0.00	2	39.50	785.2	1.14	3.84	0.27*	76.03	2.07*
Raft	0.98	2	48.97	1544	0.68	2.17	0.44*	372.6*	2.62*
Cedar	8.96	1	75.75	296.5	0.42	1.94	0.03	43.98	nd
Quesnel R.	15.70	1	47.81	1061.3	1.30	2.00	0.27	86.89	2.59

Note: The relatively unimpacted Abbott and Whiffle sites in the Main Basin of Quesnel Lake are treated as reference sites. An asterisk (*) indicates significant difference between the impacted (n = 2) and reference (n = 4) sites. A tilde (~) next to a metal header indicates a significant correlation between distance and predator metal concentration.

Manganese is found to be highest in predators from the Raft site in the West Basin, with a mean of 373 mg/kg which exceeds all other sites by a factor of five. This result is inconsistent with manganese concentrations in other sample types and should be interpreted with caution. As with other environmental samples, selenium is found to be highest at the Polley Lake site, though this difference is not significant when compared to the reference group. Arsenic, which is found to be elevated in the West Basin by all other sample types, is not significantly different between impacted and reference sites for predator invertebrates, and those data do not regress significantly with distance. Copper concentration at the Cedar site (75.75 mg/kg) is elevated relative to all other locations, but this difference cannot be considered significant because n = 1.

2.3.3 Spatial Distribution of Metals in Biofilms

A more direct spatial analysis reveals that for several metals, there is a strong relationship between distance from the spill site and analyte concentration in biofilm. This is particularly true for three metals found to be correlated (Appendix B); copper, arsenic, and lead. Figure 2.5 plots these metal concentrations in biofilms against distance from the location where Hazeltine Creek enters Quesnel Lake. Sites against the hydraulic gradient (to the east) are given negative distance values, and those along the hydraulic gradient (to the west) are given positive values. Polley Lake, representing an environment extremely impacted by tailings, is given the distance 0 on this scale (See Appendix A). This analysis shows that the concentration of metal in tile and buoy-grown biofilms tends to be highest near-to and down-lake of the spill site.

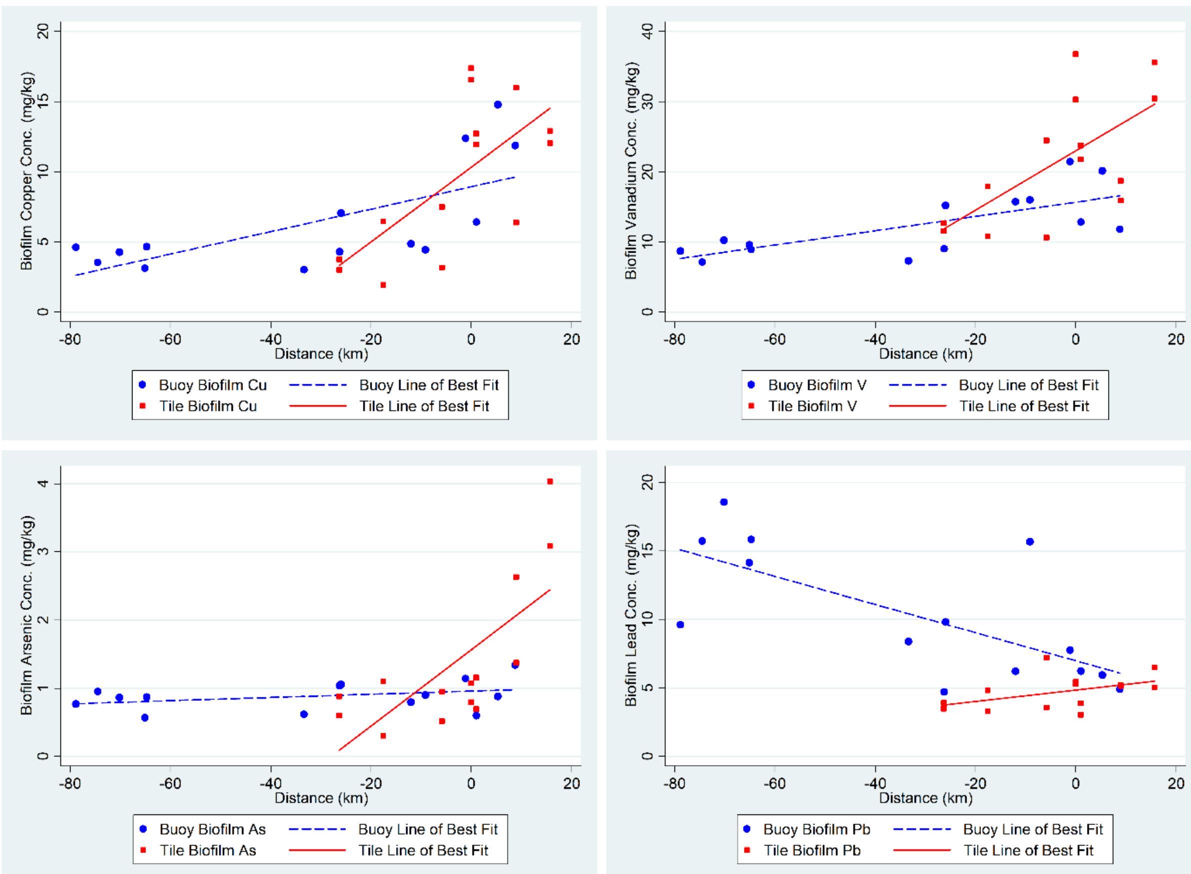

Figure 2.5 – Metal concentrations in biofilm grown on submerged plastic buoys and clay tiles, throughout the West Basin, Main Basin, and East Arm of Quesnel Lake. Linear least squares best fit lines are shown for buoy and tile-grown biofilms. Negative distance values are up-lake, east of the spill while positive distances are down-lake, west of the spill, with 0 distance representing the point where Hazeltine Creek enters Quesnel Lake.

Statistical analysis of the tile-grown pattern by linear regression and adjusting the standard error to account for repeated measures, confirms there is a significant effect of distance on trace metal concentration in tile-grown biofilm for copper ($R^2 = 0.474$, $p = 0.002$, $n = 13$), vanadium ($R^2 = 0.429$, $p = 0.007$, $n = 13$), lead ($R^2 = 0.224$, $p = 0.045$, $n = 13$), and log-transformed arsenic ($R^2 = 0.534$, $p < 0.001$, $n = 13$).

If the same type of spatial analysis (without repeat measures) is applied to the buoy-grown biofilm, significant relationships are found between distance and buoy-grown biofilm concentrations of copper ($R^2 = 0.465$, $p = 0.007$, $n = 14$) and vanadium ($R^2 = 0.512$, $p = 0.004$, $n = 14$). However, buoy-grown biofilm arsenic does not regress significantly with distance ($p = 0.212$, $n = 14$). Lead is a unique case because unlike other metals, it regresses negatively with distance ($R^2 = 0.489$, $p = 0.005$, $n = 14$), which is contrary to the pattern of tile-grown biofilms but similar to the spatial pattern of lead in sediment.

2.3.4 Isolating the Signal from Mt. Polley Spill Impacts

Although there are natural inputs of trace metals to Quesnel Lake (Appendix C), certain identifiable signals can be used to differentiate Mt. Polley spill impacts from background metal patterns. Specifically, spilled tailings are low in nickel and chromium relative to pre-spill Quesnel Lake sediments, and highly elevated in copper and vanadium (E. Petticrew, unpublished data, 2016). In spilled tailings sampled from Hazeltine Creek, the ratio of vanadium to nickel is approximately 15 (MPMC, 2015a), and V/Ni values in biofilm approach that level at sites nearest to the spill source (Figure 2.6).

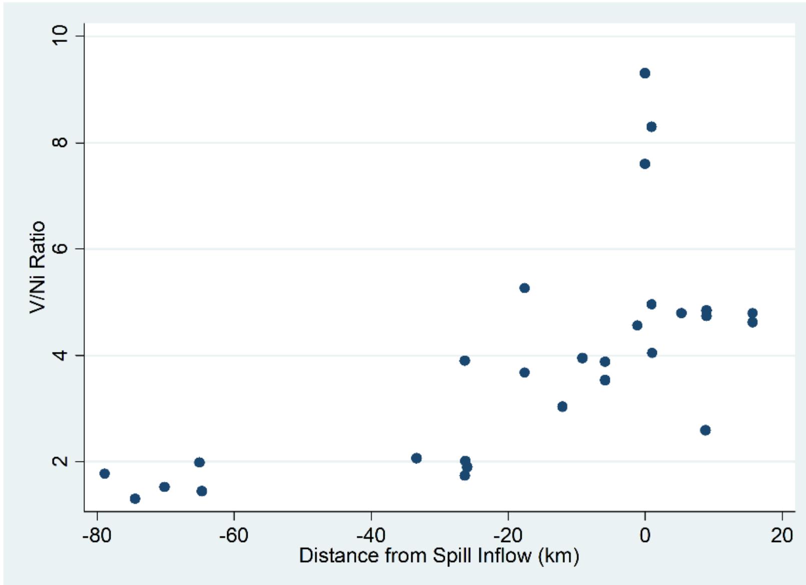

Figure 2.6 – The ratio of vanadium to nickel (V/Ni ratio) in tile and buoy-grown biofilms plotted against distance from the spill site. This ratio of elemental concentrations can be used as a tracer for the influence of Mt. Polley tailings material, which has a V/Ni ratio of ~15.

The lowest V/Ni ratios are found in the East Arm and in the Main Basin, corresponding with distances from -80 to -20 km. In those least-impacted areas the V/Ni ratio is usually around 1 to 2. At distances from -20 to +20 km, corresponding with the West Basin and spill deposit, the ratio is usually between 3 and 5. In Polley Lake and the Hazeltine bay, at sites very near 0 km distance, the V/Ni ratio spikes to its highest values between 7 and 10.

2.4 DISCUSSION

The elevated signal of copper and vanadium in sediment, biofilm, and some invertebrates in proximity to the mine site suggests the expected source of the metals is the Mt. Polley spill material. It is possible that the spike observed in copper and vanadium is also present in other spill-associated metals, including lead or manganese, but on a smaller scale, or in concentrations that require more sensitive analyses. Not only do the sediment data show that trace metal concentrations are often highest in the West Basin and within the spill deposit, but the elemental attributes of spill material are found in biological samples.

Mt. Polley spill impacts can be identified by a high ratio of copper and vanadium to nickel and chromium. In naturally occurring suspended sediments sampled in 2017 from the Mitchell, Horsefly, and Niagara Rivers (the three main tributaries to Quesnel Lake), this ratio was not found to exceed two. The ratio of vanadium to nickel was similarly low in bed sediments of creeks flowing into the West Basin of Quesnel Lake; between one and three in sediments of Poquette, Cedar, Winkley, and Edney creeks (E. Petticrew and T. French, unpublished data, 2019). The ratio of vanadium to nickel (V/Ni ratio) is employed as a tracer technique to identify the distribution of spill material, and as with the analysis of copper and vanadium, the data are indicative of a point-source of elevated metals in the West Basin. The concentration spikes of copper and vanadium in the West Basin can be attributed, at least in part, to the influence from the Mt. Polley spill, but for other metals including lead, arsenic, and selenium, the situation is more ambiguous.

The pattern of lead in Quesnel Lake contains a number of unexplained relationships which merit further investigation. One might expect lead should be of great concern to Quesnel Lake ecosystem, because of the quantity of it released by the Mt. Polley spill, which was over 100 tonnes (Environment and Climate Change Canada, 2017). However, the concentration of lead in released sediments is not especially high (MPMC, 2015a), and suspended sediments in the post-spill environment have been shown to contain mainly inert and sediment-bound lead, tending not to be immediately bioavailable (Sussbauer, 2017). In this study, when analyzing only tile-grown biofilms from Quesnel Lake, lead is found to be significantly higher in the West Basin.

However, buoy-grown biofilms sampled over a larger distance show the opposite pattern (Figure 2.6).

High lead concentrations in the East Arm may be due to the Niagara River, which drains ice fields in the Cariboo Mountains to the east of Quesnel Lake. This river contributes fine glacial sediment, which expresses itself in higher turbidity in the East Arm, relative to other areas of Quesnel Lake. Dr. B. Laval (pers. comm., 30 Sep 2017) measured turbidity (using a SeaBird SBE19 CTD) in the range of 0.5 – 1.5 NTU in the East Arm epilimnion in the summers of 2015 and 2016, and turbidity of < 0.5 NTU in the epilimnion at the junction of the three arms. However, direct sampling of suspended sediments from the Niagara River plume in spring 2017 show the lead content in those sediments was 18.9 mg/kg dry weight (E. Petticrew, unpublished data, 2017), which is within the range of sediments sampled from the West and Main Basins (Table 2.3), and lower than the CCME interim sediment quality guideline for total lead, which is 35.0 mg/kg (CCME, 2018). The high lead values in East Arm buoy biofilms remain unexplained, but may be an artefact from the longer growth period, differing depths, or metal hardware used with buoys. Further study, especially over the long term, may be required to understand the effects of the Mt. Polley spill on lead cycling in Quesnel Lake.

In addition to the 2014 tailings spill which resulted in a large benthic tailings deposit in the West Basin, the Mt. Polley Mine is permitted to discharge its mineral processing wastewater to Quesnel Lake and has been doing so since December 2015. Their permit allows for 52,000 m^3 of discharge per day, directly into Quesnel Lake near Hazeltine Creek at 50 m depth (Ministry of Environment, 2017). Because this study finds several metals elevated in the West Basin, it would be worth exploring whether the tailings deposit, the wastewater discharge, or a combination of the two, is responsible for this pattern. As explored in Table 2.1, biofilm monitoring as performed in this study has a limited ability to distinguish between various sources of metal discharge. However, there are spatial, seasonal and elemental clues which allow for some inferences.

The most important evidence which may distinguish which source is most influential on biofilm metals content is the spatial pattern. A spike is found in the West Basin for copper and vanadium, but its source is not at exactly 0 km distance, but widely extended, spread around -5 to 15 km distance. The tailings deposit in the West Basin is not isolated at 0 km distance, but extends over a large area corresponding with distances of approximately -2 to +3 km. This pattern appears to implicate that the tailings deposit is spatially related to increased metal concentrations. Another piece of evidence is that Polley Lake, designated 0 km distance, is only subject to the tailings release of 2014, and is upstream from the site where processing wastewater is released into Quesnel Lake. The Polley Lake site frequently had the highest concentrations of trace metals in environmental samples, including copper and vanadium in biofilm.

Another pattern that may indicate whether the mine spill or discharges are largely responsible for elevated metal concentrations in sampled biota is seasonality. Figure 2.3 shows how copper, vanadium, arsenic, lead, and aluminum, are elevated in the spring relative to the summer. Publicly available discharge data (MPMC 2018), compiled by A. Hamilton (Pers. Comm., 2019), shows that mean discharge from the mine in 2016 was higher during the summer sampling period (0.44 m^3/s, st. dev. = 0.02 n = 3) than during the spring sampling period (0.36 m^3/s, st. dev. = 0.12, n = 5), so the seasonal change does not appear to be related to wastewater discharge. There is reason for the benthic tailings to contribute more trace metals to the water column during the spring. This is because of lake overturn, which at the least would allow for the spread of dissolved metal ions through the vertical profile, and if the disruption is great enough, would resuspend fine tailings particles. Higher concentrations of metal in the spring could also be related to tributary inputs during freshet, but other evidence (Appendix C) suggests those stream inputs may have a small influence on biofilm trace metal concentrations when compared to the tailings deposit. There is some evidence of this spring resuspension, in the form of downstream turbidity and suspended copper in the spring following the spill (Sussbauer, 2017). The seasonal and spatial patterns appear to indicate that the tailings deposit has a greater influence on bioavailable metals than does the ongoing discharge of wastewater, though none of these conclusions can be made certain without follow-up study.

It is difficult to assess how harmful the reported quantities of trace metals in biofilm would be to higher organisms, such as invertebrates that feed on biofilm, but there are indications from the literature they present some risk. In a study by King et al. (2005), the authors exposed *Melita plumulosa*, an estuarian amphipod species, to differing amounts of dietary copper in algae. When feeding *M. plumulosa* algae containing 100 mg/kg Cu (wet weight), they found a mean copper accumulation efficiency of 33%, which was much higher than the accumulation rate of sediment at 7.8%. Biofilm copper values from Quesnel Lake, expressed as dry weight are ~5 mg/kg in the Main Basin and ~15 mg/kg in the West Arm. Though these concentrations are numerically lower than those from King et al. (2005) biofilm samples from Quesnel Lake averaged 88% water weight, meaning the concentrations of copper per wet weight would be approximately 9x higher, in the range of 45 - 135 mg/kg wet weight, into which falls the 100 mg/kg concentration tested by King et al. (2005). The King et al. study has two implications for Quesnel Lake. First, biofilms in Quesnel Lake possibly contain high enough concentrations of copper to affect higher organisms like amphipods, which are found in abundance in Quesnel Lake, and secondly, biofilm has the potential to be a large-scale vector for metals into Quesnel Lake area biota.

Elevated concentrations of trace metals including copper and vanadium, and possibly others, can potentially affect salmonid health at several life stages. The Salmonidae taxonomic family encompasses anadromous salmon stocks protected by the DFO Fisheries Act, as well as Coho salmon (*Oncorhynchus kisutch*) and bull trout (*Salvelinus confluentus),* which are COSEWIC listed fish populations in Quesnel Lake (COSEWIC, 2012, 2016). Most other species of interest to anglers and tourists in the region, such as rainbow trout (*Oncorhynchus mykiss*) and kokanee (*Oncorhynchus nerka*), are also salmonids. Exposure to elevated levels of copper, selenium, and vanadium presents a risk to developing salmonids, potentially altering behavior (Campbell et al., 2002; Schiffer, 2016), increasing the incidence of deformities (Jezierska et al., 2009; Sfakianakis et al., 2015), reducing spawning success (Rudolph et al., 2008), and reducing growth (Hansen et al., 2009; Hilton and Bettger, 1988; Lundebye et al., 1999). If the bioaccumulation of copper and trophic biomagnification of selenium continue up the food web to organisms that prey on invertebrates, then salmonids in Quesnel and Polley Lakes are at risk of developing elevated body loads of these metals, relative to other environments.

Other studies of Quesnel Lake fish and invertebrates have not found that trace metal concentrations in the West Basin are lethal (G. Pyle, pers. comm., 18 May 2017; L. Williston, pers. comm., 16 Jun 2016). However, the elevated uptake of metals in West Arm biofilm indicates a higher concentration of bioavailable metals in that region, making sublethal effects possible, at least at some scales. Sublethal impacts to salmonids represent a potential threat to populations of coho salmon (*Oncorhynchus kisutch*) and bull trout (*Salvelinus confluentus*), which are respectively designated as Threatened, and Special Concern by the Committee on the

Status of Endangered Wildlife in Canada (COSEWIC, 2012, 2016). Other populations of fish in Quesnel Lake, including rainbow trout (*Oncorhynchus mykiss*) and sockeye salmon (*Oncorhynchus nerka*) are also threatened by Mt. Polley trace metals impacts, translating into potential impacts to fisheries of recreational, Aboriginal, and economic importance.

2.5 CONCLUSION

It was hypothesized that the Mt. Polley spill deposit and ongoing wastewater discharge are exerting a chemical influence on impacted water bodies in the form of increased concentration of bioavailable trace metals, and that this chemical influence would be identifiable in environmental samples as a spatial pattern. Binary comparison of differently impacted water bodies, as well as spatial analysis along a continuous distance variable, return significant results for copper and vanadium concentrations in environmental samples, and show that metal concentrations decrease with increasing distance away from the spill, especially in an up-lake direction. Some testing parameters find significantly increased lead and arsenic concentrations associated with the spill, while other parameters find no significant effect, or in the case of lead, significant but opposite effects.

Other objectives of this study were to distinguish between natural and anthropogenic sources of trace metal, and to distinguish between contamination from the tailings deposit and that from ongoing wastewater discharge. Elevated vanadium and copper levels are believed to be spill-associated, and the V/Ni ratio can be used to trace and distinguish spill material from other sources of metal. When applied to biofilm samples, this tracer indicates that a point source of metal contamination exists at Hazeltine Creek, and the influence of this source attenuates over the scale of tens of kilometers. A seasonal pattern, with some metals elevated in the spring, may be a result of lake overturn increasing the mobility of the spill deposit. While not conclusive, this may indicate that the tailings deposit has more of an influence on trace metal availability than discharge. The Mt. Polley spill does appear to have a spatial pattern of contamination which is identifiable by biomonitoring.

3 Trophic and Food Web Transfers of Trace Metals

3.1 Introduction

The Mt. Polley Mine spill of 2014 released a large volume (~ 20 M m^3) of trace-metals rich tailings (water and solids) and other sediment into the West Basin of Quesnel Lake, BC, Canada (Petticrew et al., 2015). The tailings were processed at Imperial Metal's Mt Polley Mine to a median grain size of 50 µm. This mine spill, explained in greater detail in section 1.1, left a 5-10 m thick layer of both coarse tailings and fine mineral rich deposits in the West Basin of Quesnel Lake. Deposition of the coarser fraction of spill material occurred within days, while finer spill materials, which had a mean particle diameter below 1 µm, remained in a plume in the water column for months (Petticrew et al., 2015). Initial measurements showed that the spilled tailings were found to be elevated in copper, iron, manganese, and arsenic in excess of the Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines for the protection of aquatic life (MPMC, 2015a).

Given the high trace metals content, large volume, and extensive surface area of the spilled materials deposit, the potential for trace metals pollution of Quesnel Lake biota is a concern for local communities and regulatory authorities. This lake is habitat for nationally important anadromous salmon stocks, and two protected fish populations; Bull Trout and Coho salmon, which are listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2012, 2016). Additionally, the lake is the source of drinking water for the nearby town of Likely, and is within the un-ceded traditional territory of the Secwepemc (Shuswap) First Nation, with two bands, T'exelcemc and Xatśūll, heavily impacted by the spill.

The physical impact of the spill is relatively well understood, and described elsewhere (Morgenstern et al., 2015; Petticrew et al., 2015), but the biogeochemical effects of the spill are poorly understood, with major questions unanswered about the bioavailability of spill materials, the potential for bioaccumulation and biomagnification, and the long-term outlook of the aquatic ecosystem. To help answer these questions, biomonitoring has been employed as a way of exploring the movement of trace metals into and through the lowest levels of the food web. Biomonitoring involves using biota, usually naturally occurring, as a way of testing directly for the presence and concentration of environmental contaminants (Carter et al., 2006). It is an appealing tool because it avoids many of the uncertainties and complications that come from aquatic ecotoxicology, and tests organisms directly (Bonada et al., 2006). It is usually biota that are of greatest legal and managerial concern after environmental damages occur, especially given their connection to human health and well-being through diet, recreation and employment generation. Using the approaches described above they can be examined directly.

As explored in the prior chapter (Chapter 2), there are criteria to determine if a particular organism is a good biomonitor, which are presented by Bonada et al. (2006). It is generally understood that in aquatic environments, invertebrates and biofilm (autotrophic microbial assemblages) make good biomonitors because of their ubiquity and ability to sequester contaminants including persistent organic pollutants, petrochemicals, and trace metals (Kohušová et al., 2011). Invertebrates are particularly well suited to biomonitoring of the Mt. Polley spill, because of the ability to subdivide invertebrates into meaningful groups that provide additional information about trophic level and possible food web transfers (Farag et al., 1998). This additional layer of information indicates the extent of trace metals transference into the food web, and the potential fate of trace metals that do enter the food web.

The classification of littoral invertebrates by their morphology, which reflects their feeding habits, allows trophic pathways to be investigated. Aquatic invertebrates in this study are classified into one of four well established functional feeding groups which are defined by organismal morphology, as first developed by Cummins and Klug (1979). The feeding groups utilized by this study are: scrapers, which feed by scraping and consuming epilithic biofilm from their environment; filterers, which have specialized appendages or create silk nets to capture suspended or dissolved organic matter; predators, which feed on other heterotrophs; and collectors, a generalist group which gathers organic matter from places where it is accumulated by fluvial processes. The feeding ecology of these groups, in conjunction with their metals content, provides evidence of where and how metals are transferred in the lower level of the food web. A particularly interesting link in the food web is between biofilms and scrapers, which is a theoretical pathway for metals to enter the food web (Besser et al., 2001).

The movement of trace metals in an aquatic food web is dependent on the metal in question and many environmental factors. Organisms can sequester metals both from environmental exposure and from dietary pathways (Campbell et al., 2002). There are two patterns of metal accumulation that are of interest regarding the Mt. Polley spill. First is bioaccumulation, which is a process in which an organism accumulates a substance, usually a contaminant, faster than it is able to metabolize or excrete it. Bioaccumulation is common and well documented in aquatic environments contaminated by copper, zinc, cadmium, and lead (Goodyear and McNeill, 1999). Bioaccumulation in aquatic invertebrates has also been identified for iron and aluminum (Winterbourn et al., 2000), vanadium (Chiffoleau et al., 2004) and cadmium (Cain et al., 2011), among many other trace metals.

A potentially more concerning pattern of metal uptake in the aquatic environment is biomagnification, in which a material becomes increasingly concentrated in organisms at higher levels in the food web (Streit, 1992). This is usually a result of contaminants that are lipophilic and persistent. These contaminants are passed along through the food web without being broken down or excreted. Higher trophic organisms tend to be long-lived, and rely on a large base of producers and primary consumers, so magnifying pollutants can accumulate to dangerous levels (Kidd et al., 1998). Apex predators, such as birds of prey and large fish, can be susceptible to toxic levels of biomagnification, but the patterns are often also visible at lower trophic levels (Mason et al., 2000). Most trace metals, including cadmium, copper, iron, lead, vanadium, and zinc do not biomagnify under ordinary environmental conditions. Some trace metals do commonly biomagnify, however. Of great interest to the investigation of the Mt. Polley spill are selenium and mercury which are both known biomagnifiers (Ouédraogo et al., 2015), as they are a potential threat to Quesnel Lake biota. Selenium is highly elevated in spill materials relative to background (Figure 1.2), so the potential biomagnification of selenium is an avenue of harm to the biota in Quesnel Lake that is worth investigating.

Stable isotopes of nitrogen can be used to better understand trophic positioning of organisms. The ratio of nitrogen isotopes ^{14}N and ^{15}N can determine the trophic level of an organism, as the heavier isotope is retained in 3-4 parts per thousand higher concentration with each trophic transfer, from a consistent isotope ratio in the atmosphere (Peterson and Fry, 1987). Organisms at higher trophic levels in aquatic systems tend to concentrate ^{15}N, and the ratio of this isotope can be used to infer trophic positioning along with published isotopic discrimination factors for different organisms.

It is hypothesized that organisms in water bodies affected by the Mt. Polley spill are taking-up metals through environmental exposure, and also through their diet, in a manner distinct from unimpacted water bodies. Specifically, biofilm is hypothesized to be a trace metals vector, adsorbing metals from the water column and making them available to higher organisms through dietary exposure. Of those metals associated with the spill (Appendix B), copper and vanadium are hypothesized to be bioaccumulating, while it is hypothesized that selenium may be biomagnifying in biofilm and invertebrates. The null hypothesis is that trophic patterns, including bioaccumulation or biomagnification, are not different between impacted water bodies and references. Additionally, it is hypothesized that taxonomic compositions of invertebrate communities differ between differently-impacted environments, with a null hypothesis that invertebrate abundance is unrelated to environmental concentrations of spill-associated metals, and does not differ between impacted and reference sites. These hypotheses will be tested by using food web diagrams and stable isotope ratios of nitrogen to assess the concentration and transfer of metals in a simplified aquatic food web, and by relating insect abundance with concentrations of vanadium, copper, and selenium in the environment.

3.2 METHODS

This study of invertebrates and biofilm was done with a focus on the West Basin of Quesnel Lake, which contains a very large volume (~20 M m^3) of spill materials (Petticrew et al., 2015). Fourteen sites were sampled in the spring and summer of 2016 during two sampling periods (Figure 2.1). The first sampling period took place in spring, April 13 to May 26, and the second, later in summer, June 2 to July 14. Each sampling period was six weeks, a period of time designed to allow adequate growth of biofilm. Twelve custom-made unglazed clay tiles of 225 cm^2 surface area each (2700 cm^2 total) were placed at each of the fourteen sites as artificial substrates to allow for growth of native epilithic biofilm communities. In streams, biofilm tiles were anchored to cement blocks and placed in pool reaches of 30 to 100 cm water depth. In Quesnel Lake, the 12 tiles were attached to moorings at 0.3 to 3.6 m depth, with 30 cm spacing between tiles. Moorings were placed in 15 m deep water, near the shore of Quesnel Lake (usually within 200 m). At the end of the sampling period, biofilms were scraped, frozen, lyophilized and stored for analyses.

An array of DGT samplers was deployed at the same sites as biofilm tiles, for one week in the middle of each sampling period. DGT samplers were purpose made using chelex and Fe-based resins by researchers at the Trent University Laboratory of Aquatic Sciences and Biogeochemistry, as described by Shi et al. (2016). Deployment took place on day 21, and retrieval on day 28, of the 42-day period. In tributary streams and in Polley Lake, the samplers were attached to wooden stakes and placed approximately 50 cm below the water surface, and at least 25 cm above bed sediment. In Polley lake, DGTs were hung on biofilm moorings at 1.95 m depth (i.e. in the middle of the 12 biofilm tiles). Samplers were left for one week, as longer deployments can cause the membrane (Figure 1.4) to become fouled, making the devices inaccurate (Turner et al., 2012). Following deployment in Quesnel Lake, DGT samplers were returned to Trent University, disassembled, and the binding resin was analyzed by ICP-MS (Thermo Scientific XSeries) at the Trent University Water Quality Centre, the accuracy of which was assessed using SLEW-3 and SLRS-5 certified reference material. The resultant DGT data represent concentrations of dissolved, cationic metals in sampled water bodies.

Additionally, during each six-week sampling period, invertebrates were sampled five times, on a weekly basis. A kick net sampler with 800 μm mesh size and a 460 x 230 mm frame was used for invertebrate capture, followed by field elutriation in acid-washed buckets, and storage of invertebrate samples in sterile 50 ml sample tubes. At each site, substrate was perturbed by kicking/stomping while walking backwards at a rate of ~0.5 m/s, repeated three times for one minute each. This occurred on the 7th, 14th, 21st, 28th, and 35th days of the sampling period. The five invertebrate samples from the sampling period were combined to obtain sufficient volumes for analysis. These samples correspond spatially and temporally with the biofilm sampling, although efforts were taken not to affect the environments containing biofilm growth tiles. These population sampling methods are not absolute population measures, but represent catch per unit effort (CPUE), based on one-minute kick-net sampling. CPUE is an imperfect but useful measure which corresponds with population abundance (Harley et al., 2001).

Invertebrates were separated into one of four functional feeding groups: scraper, collector, predator and filterer, based on physiological features and following a key by Merritt and Cummins (1996). Of these four feeding groups, the filterer feeding group was found to be very rare in Quesnel Lake, and was dropped from many analyses for lack of sample availability. Generalist feeders such as many Amphipoda and Plecoptera were designated as collectors. In addition to the feeding groups, 13 common taxonomic groups, listed in Table 2.2, were counted in these sampled invertebrates, using another key from Hauer and Resh (2007). Oligochaeta, which are small (<1 mm) and difficult to identify based on morphology (Seesao et al., 2017), were identified only to phylum. Most other invertebrates were identified to order, and the order Diptera was subdivided into three families. The functional feeding and taxonomic classifications were done parallel to one another, and most taxonomic groups do not correspond with a specific functional feeding group.

Following invertebrate identification, samples of invertebrates and biofilm were lyophilized, then homogenized. Following Steinman et al. (2007), small subsamples of invertebrate and biofilm homogenate had their ash-free dry mass measured by weighing them before and after spending 60 minutes in a muffle oven at 500° C. For metals testing by inductively coupled plasma mass spectrometry (ICP-MS), subsamples of 0.5 g were taken, and acid digested using a mixture of 3.5 mL nitric acid and 1.5 mL hydrochloric acid, then heated in a block digester and held at 90° for seven hours, using a modified version of EPA Method 3051A (Link et al., 1998). Samples were then matrix matched and run through an ICP-MS unit at the UNBC Northern Analytical Laboratory Service (Agilent Technologies 7500cx Series). Data were returned in μg/g for 32 metals, nonmetals, and metalloids, including copper, vanadium, aluminum, selenium, iron, lead, mercury and manganese.

Nitrogen isotope ratios, used for quantification of trophic position, were also measured by isotope ratio mass spectrometry (IRMS). This is done by taking a small (~50 μg) subsamples from the biofilm and invertebrate homogenates, which were inserted into tin capsules. At the University of California - Davis Stable Isotopes Facility, these samples were put through a continuous-flow isotope ratio mass spectrometer, which distinguishes the ratio of carbon and nitrogen isotopes (Spötl and Vennemann, 2003). These data were synthesized into the enrichment of the stable nitrogen-15 isotope ($\delta^{15}N$), relative to known standards.

To allow for the direct quantification of trophic levels, relative stable isotope enrichment in the food web was performed following a modified version of the method in Hobson et al. (1995). Trophic levels of all invertebrate samples were quantified using their $\delta^{15}N$ value, in reference to biofilm, which is treated as a primary producer (trophic level 1) with a mean $\delta^{15}N$ of 1.269 and standard deviation of 1.516. For invertebrates, Caut et al. (2009) report a generalized isotopic discrimination factor of 2.5 parts per thousand (‰) per trophic level, leading to equation

3.1, in which $TL_{organism}$ indicates trophic level, 1.269 is the mean $\delta^{15}N$ of biofilm, 1 is the trophic level of biofilm, and 2.5 is the isotopic discrimination factor.

$$TL_{organism} = 1 + \frac{\delta^{15}N_{organism} - 1.269}{2.5}$$

Equation 3.1

In reality, biofilm is a multi-species, multi-trophic assemblage, so the derived trophic levels calculated by this equation are likely to be low in absolute terms and are only useful in comparison to each-other.

To assess the movement of metals in the food web, and also to quantify links between the abiotic environment and the food web, “transference factors” (TF) are used, which are based on the ratio of analyte concentrations between two materials in the environment. This measure is often referred to as a “biomagnification factor” in food web studies (Franklin, 2016), but this name is not appropriate in the context of Mt. Polley research, because most metals associated with the spill do not biomagnify. The notable exception to this trend is selenium. The calculation in Equation 3.2 is used to assess transfer of metals in the environment generally, from sources including water and sediment, to potential sinks which are organisms:

$$\mathrm{TF} = \frac{[\mathrm{analyte}]_{\mathrm{sink}}}{[\mathrm{analyte}]_{\mathrm{source}}}$$

Equation 3.2

Transference factors (TF) lower than one indicate that metals have higher concentrations at their source, while values greater than one indicate that concentrations are magnified at their sink (Kim and Kim, 2016). The TF index is used to assess the relationship between individual links in the environment but cannot be used to assess magnification or transfer in the food web as a whole. For instances where a relationship is found between overall trophic level and analyte concentration, the trophic magnification factor (TMF) - sometimes referred as a food web magnification factor (Hop et al., 2002) - is employed to quantify the extent of trophic biomagnification across the entire food web.

Quantification of trophic magnification is done using calculations shown in Equations 3.3 and 3.4 (below), which are adapted from Borgå et al. (2012) by changing the trophic level reference and isotopic discrimination factor. In this method, analyte concentrations are log transformed and plotted against trophic level (Figure 3.1) to match available Quesnel Lake data. Log transformation is appropriate because biomagnification is often exponential in aquatic food webs (Franklin, 2016). A linear regression is plotted for this relationship, in which m = slope, TL = trophic level, and b = intercept. Following this is Equation 3.4, in which the antilog of the slope (m) from the regression is equal to the trophic magnification factor (TMF), representing the increase in contaminant concentration from one trophic level to the next, across the entire food web.

$$\log[\text{analyte}] = m*TL + b$$

Equation 3.3

$$TMF = 10^{m}$$

Equation 3.4

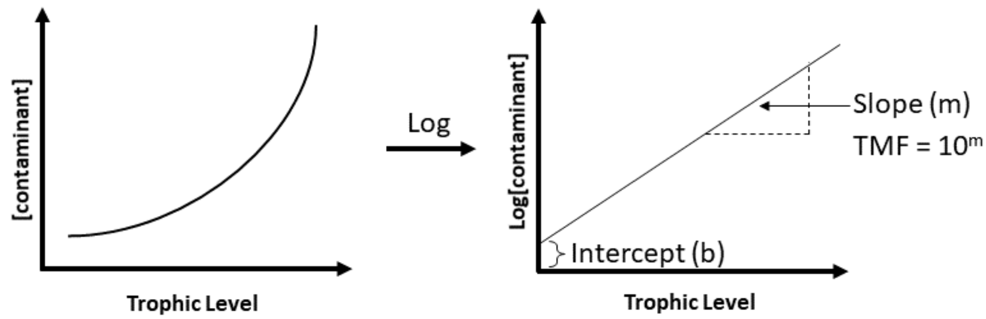

Figure 3.1 – Graphs showing how the trophic magnification factor (TMF) is calculated from the log of contaminant concentration (y axis) and trophic level (x axis), as expressed in Equations 3.3 and 3.4. Adapted from Borgå et al. (2012).

3.3 Results

3.3.1 Food Web Transfer – Correlations and Transference Factors

Metal concentration data are organized and analyzed in this section based on a simplified food web. This food web combines abiotic environmental samples (sediment and DGT-labile water measurements) with biotic samples (biofilm and three invertebrate feeding groups). In these diagrams, sites from Quesnel Lake, Polley Lake and the Quesnel River (n = 14) are considered to be potentially impacted, and are compared to paired sites (n = 14) located in inflowing environments that could not have been impacted (Figure 2.1). Potentially impacted sites exhibit statistically significant differences from the paired sites in tributary environments. The prior chapter found spatial association between the spill and organism concentrations of copper and vanadium, so these metals are targeted. Selenium has the potential to biomagnify, so is also targeted for food web analysis. These three metals are all believed to be associated with spill material (Appendix B).

3.3.1.1 Copper in the Food Web

The impacted sites' copper concentration food web (Figure 3.2A) shows strong and mostly significant correlations between sediment and biofilm, biofilm and scrapers, and scrapers and predators. This is an unbroken pathway though the food web. Copper concentrations are an order of magnitude higher in sediments than they are in organisms, with TF values between 0.027 for sediment–biofilm, and 0.121 for sediment–predators. Looking at organisms' body loads, copper concentration increases at each link in the food web with transference factors of 3.09 between biofilm and primary consumers (scrapers and collectors), and about 1.35 between primary consumers and secondary consumers (predators).

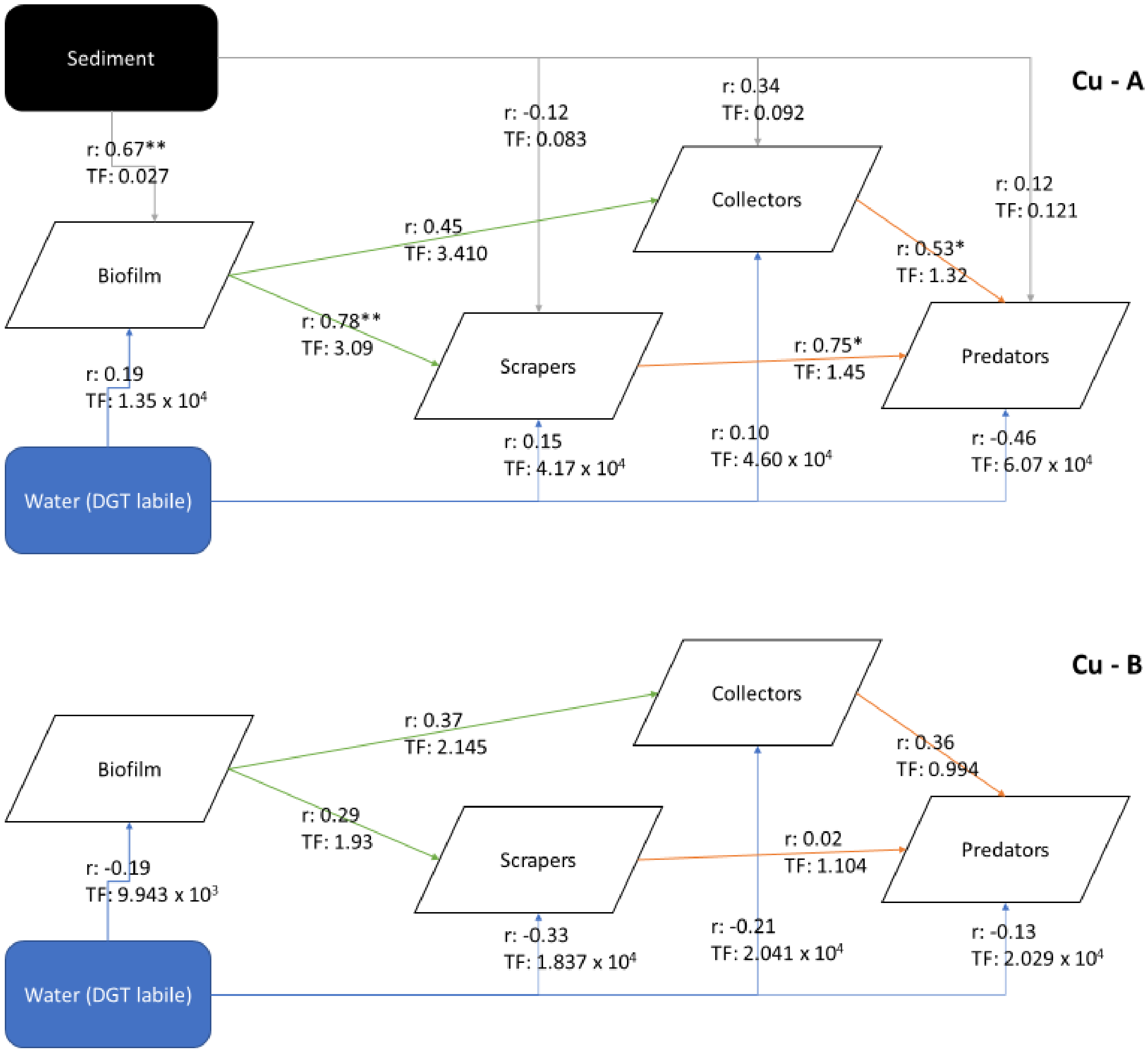

Figure 3.2 – Food web relationships of copper at sites that were potentially impacted by Mt. Polley Mine activities (A top), and at reference sites which were upstream of the spill and not impacted (B bottom). Arrows indicate metal movement between materials in the environment, and are color coded to indicate transfer from water (blue), from sediment (black), between a producer and a consumer (green), and between two consumers (orange). Values superimposed over top of arrows are Pearson's correlation coefficients (r), and transference factors (TF). Note: Near-significant correlations with $p < 0.1$ are denoted with a single asterisk (*) and significant correlations with $p < 0.05$ are denoted with a double asterisk (**).

At reference sites upstream from possible Mt. Polley effects (Figure 3.2B), correlation coefficients between materials in the environment are universally lower than at impacted sites. None of the relationships correlate significantly with each-other, which may illustrate lower trophic mobility of trace metals at these sites. Biomagnification factors are also universally lower compared to impacted sites, by about one third, and there does not appear to be trophic magnification within the invertebrate feeding groups, with TFs of ~1 at all levels.

3.3.1.2 Vanadium in the Food Web

Vanadium is strongly associated spatially with the Mt. Polley spill, and is elevated in sediments, biofilm, and invertebrates that are proximal to spill materials, as explained in Section 2.3.2. However, food web analysis (Figure 3.3A) does not indicate any particular trophic patterns of uptake at potentially impacted sites.

There are only two significantly correlated links between materials in the vanadium food web. First is sediment to biofilm at impacted sites ($r = 0.60$, $p = 0.024$). The transference factor between these materials is 0.233. Looking higher up the food web at invertebrates from potentially impacted sites (Figure 3.3A), transference factors for vanadium are all below 1. The predator functional feeding group is significantly correlated to vanadium in biofilm, though this relationship is not found in the intermediary food web transfers. Scrapers do not correlate with the metals content of biofilm, but scrapers do correlate near significantly with collector invertebrates, with a fairly high a coefficient of determination ($r = 0.067$, $p = 0.098$).

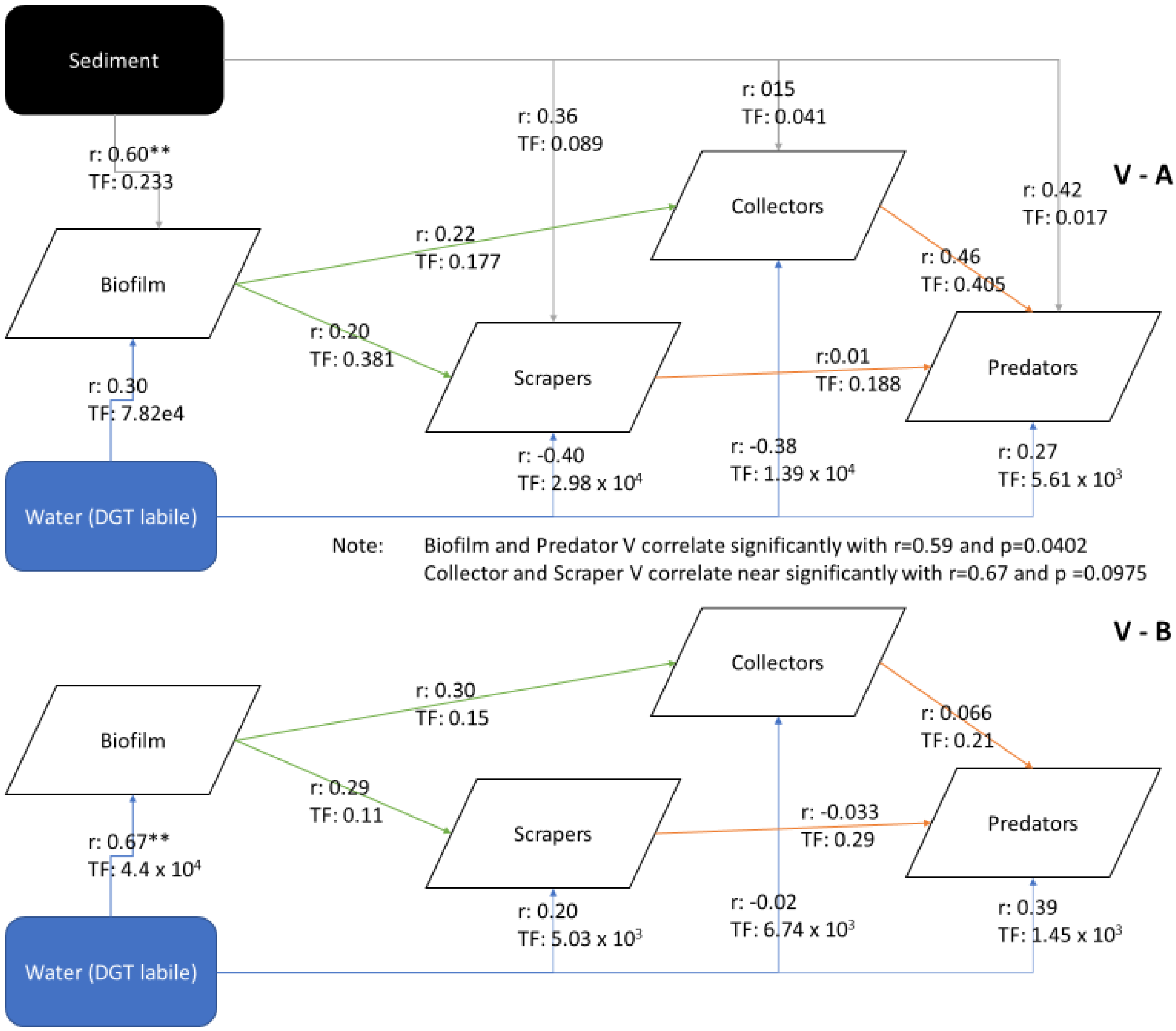

Figure 3.3 – Food web relationships of vanadium at sites that were potentially impacted by Mt. Polley Mine activities (A top), and at upstream reference sites which were not impacted (B bottom). Arrows indicate metal movement between materials in the environment, and are color coded to indicate transfer from water (blue), from sediment (black), between a producer and a consumer (green), and between two consumers (orange). Values superimposed over top of arrows are Pearson's correlation coefficients (r), and transference factors (TF).
Note: Near-significant correlations with $p < 0.1$ are denoted with a single asterisk (*) and significant correlations with $p < 0.05$ are denoted with a double asterisk (**).

At unimpacted sites (Figure 3.3B), the only identifiable relationship in the food web is between vanadium concentrations in water and biofilm ($r = 0.67$, $p = 0.012$). Transference factors between feeding groups are not universally higher at impacted sites relative to reference, but all tend to fall between 0.15 and 0.4, with high variability.

3.3.1.3 Selenium in the Food Web

At potentially impacted sites (Figure 3.4A), there is a very strong correlation between DGT-labile and sediment-associated selenium ($r = 0.93$, $p < 0.001$). Collector invertebrates at impacted sites correlate strongly and significantly with both DGT ($r = 0.82$, $p < 0.001$) and sediment ($r = 0.73$, $p = 0.003$) selenium measurements, but collectors do not correlate significantly with scrapers or predators. There is an indirect link between DGT-labile and scraper selenium, through biofilm, which is correlated with both and could be an intermediary. Higher up the food web, the collector–predator correlation is near significant ($r = 0.52$, $p = 0.086$). Transfer rates among trophic levels are relatively consistent at impacted sites, with biofilm–invertebrate TF of 8.4, and primary to secondary consumer TF of 1.5. Concentrations of selenium in sediment are approximately an order of magnitude higher than that in biofilm and invertebrates.

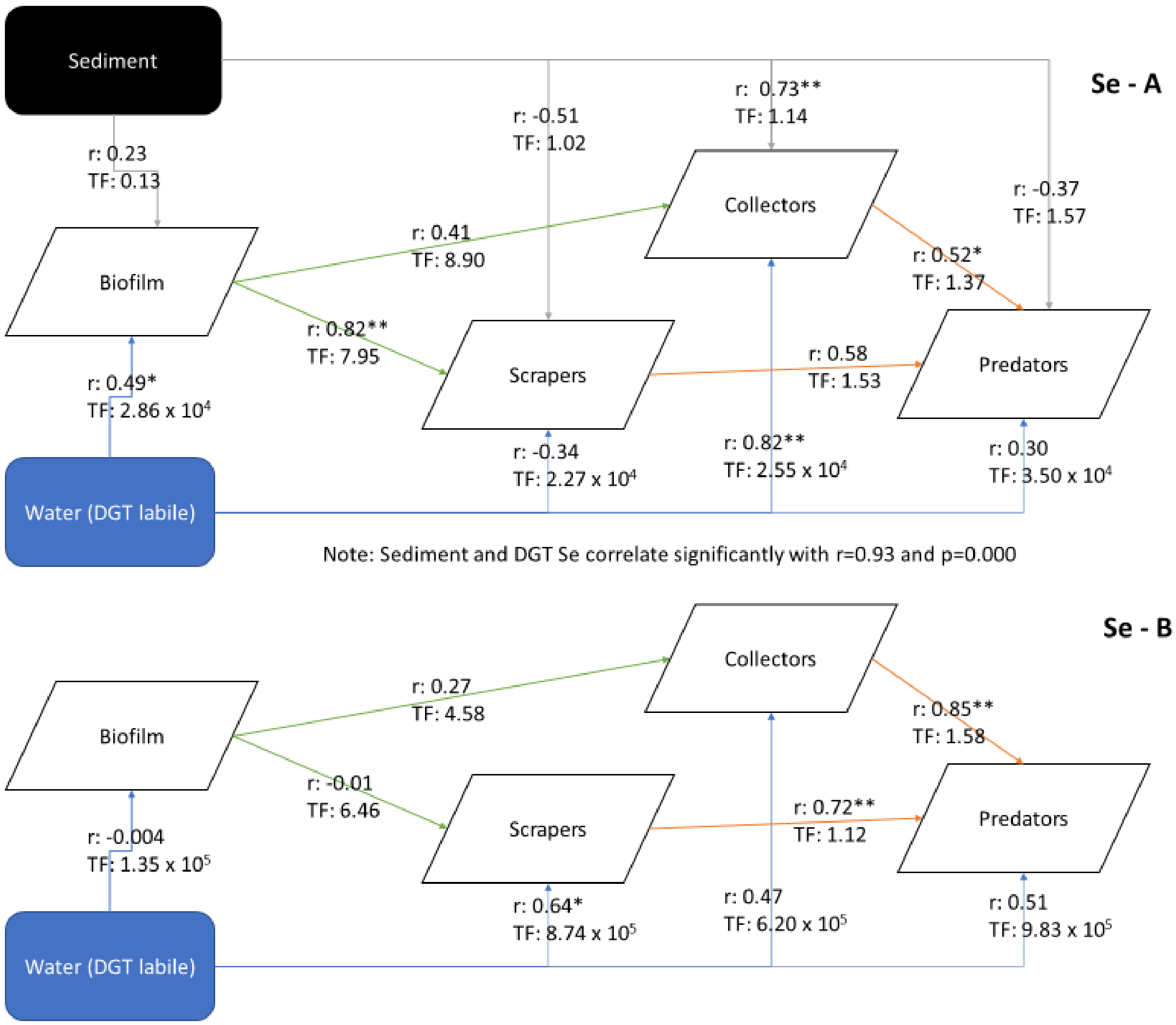

Figure 3.4 – Food web relationships of selenium at sites that were impacted by Mt. Polley Mine activities (A top), and at reference sites which were not impacted (B bottom). Arrows indicate metal movement between materials in the environment, and are color coded to indicate transfer from water (blue), from sediment (black), between a producer and a consumer (green), and between two consumers (orange). Values superimposed over top of arrows are Pearson's correlation coefficients (r) and transference factors (TF).
Note: Near-significant correlations with $p < 0.1$ are denoted with a single asterisk (*) and significant correlations with $p < 0.05$ are denoted with a double asterisk (**).

At unimpacted sites, the selenium situation is quite different, with no apparent correlation between DGT-labile and biofilm selenium, and no significant correlation between biofilm and invertebrate selenium. However, DGT selenium is correlated consistently with different invertebrate feeding groups. Scraper and collector selenium are strongly correlated with predator selenium at unimpacted sites. Transference factors are much lower at unimpacted sites, particularly between biofilm and primary consumer invertebrates, where the biomagnification factors are ~5.5 at reference sites compared with ~8.4 at impacted ones.

Although it is not represented in the flow chart, at impacted sites (Figure 3.4A), there is a strong correlation between DGT-labile and sediment-associated selenium. Selenium concentrations are also higher in invertebrates than they are in abiotic environmental samples.

3.3.2 Assessing Biomagnification using $\delta^{15}N$ Trophic Levels

When selenium concentrations in organisms from Quesnel Lake sites are plotted against trophic level, a pattern of increasing concentration with increasing trophic level can be seen. This pattern may imply there is biomagnification, and does not occur for copper or vanadium. If biofilm is included in the analyses, the relationship is strongly significant by linear regression, both in the spring ($p < 0.001$, $R^2 = 0.6976$, $n = 25$) (Figure 3.5), and also in the summer ($p < 0.001$, $R^2 = 0.438$, $n = 22$) (Figure 3.6). However, it is not ideal to use biofilm in this analysis, because in the calculation of trophic level, biofilm is used as an autotrophic reference, and has its trophic level arbitrated to equal 1 (Equation 3.1). If biofilm is excluded from analysis, and only invertebrates are included, the relationship between trophic level and selenium content remains significant during the spring sampling period ($R^2 = 0.431$, $p = 0.003$, $n = 18$), but not during the summer ($p = 0.523$, $n = 15$). The pattern between trophic level and selenium concentration is only found at potentially impacted sites, and no trophic-selenium relationship is found for upstream sites unimpacted by the spill, regardless of season or other factors.

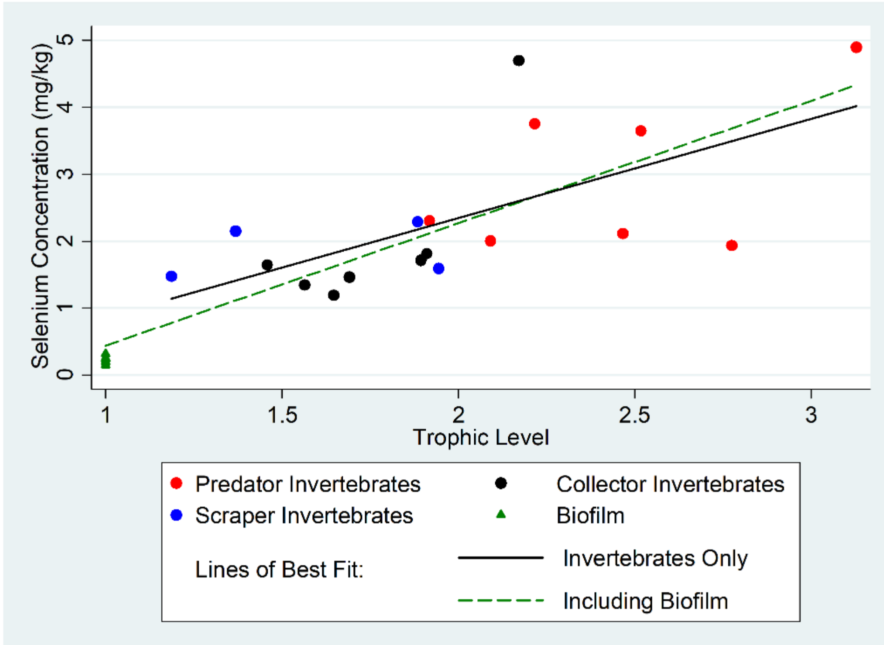

Figure 3.5 - Selenium concentrations in organisms from Quesnel Lake, Polley Lake and the Quesnel River in the spring, plotted against their trophic level as assessed by stable isotope ratios of nitrogen. Two lines of best fit are shown; one including biofilm (green), and another which excludes biofilm (black).

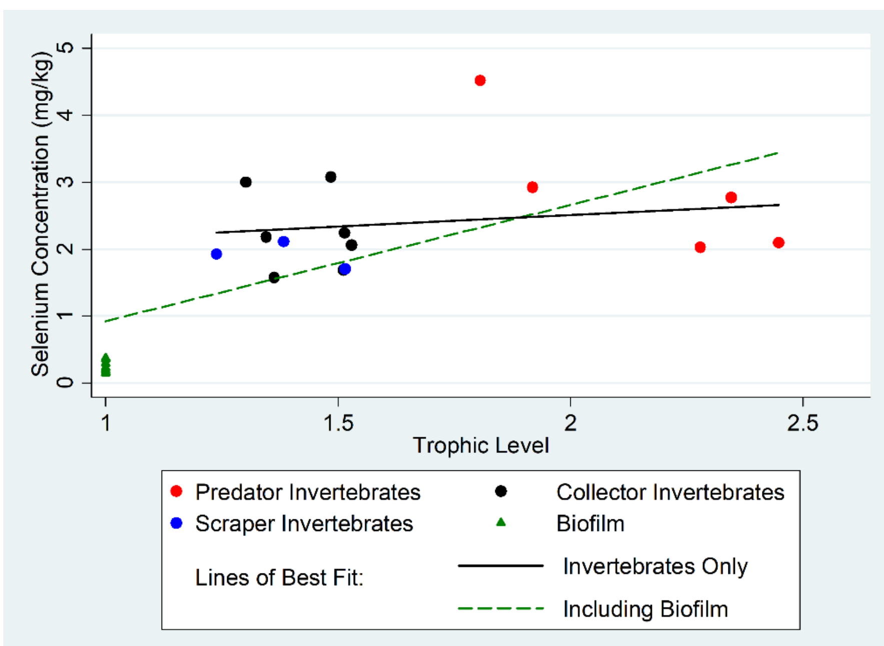

Figure 3.6 – Selenium concentrations in organisms from Quesnel Lake, Polley Lake and the Quesnel River in the summer, plotted against their trophic level as assessed by stable isotope ratios of nitrogen. Lines of best fit are shown for the entire dataset, including biofilm (green), as well as with biofilm excluded (black).

Selenium concentrations in sampled invertebrates are typically between 1 and 4 mg/kg, and in biofilm are always below 0.5 mg/kg. Following the method of Borgå et al. (2012), the invertebrates only best fit line from Figure 3.5 can be log transformed, which results in a significant linear regression with non-biased residuals (not shown). The log transformed plot is then translated into a trophic magnification factor (TMF) using Equation 3.6 below:

$$\log(\text{selenium}) = 0.245*TL - 0.159$$

Equation 3.5

$$TMF = 10^{0.245} = 1.758$$

Equation 3.6

Using this method, the trophic magnification factor of selenium was 1.758 during the spring 2016 sampling period, meaning that invertebrate samples of Quesnel Lake have a mean change in selenium concentration of 175.8% from one trophic level to the next.

3.3.3 Invertebrate Taxa and Diversity

Differences in invertebrate abundance between sites may indicate ecological effects relating to the Mt. Polley spill. These patterns associated with invertebrate catch per unit effort complement trophic analyses from the prior section, and suggest that the Mt. Polley spill may have consequences for Quesnel Lake organisms.

3.3.3.1 Invertebrate capture with time

The overall trend during the sampling periods is that more invertebrates were available for sampling in the spring, compared with the summer period (Figure 3.7). This trend is especially strong with invertebrates collected during their aquatic larval stage, prior to metamorphosis and emergence, as can be seen clearly with *Plecoptera* (stonefly) count, shown in three small creeks where this phylum was commonly found (Figure 3.8).

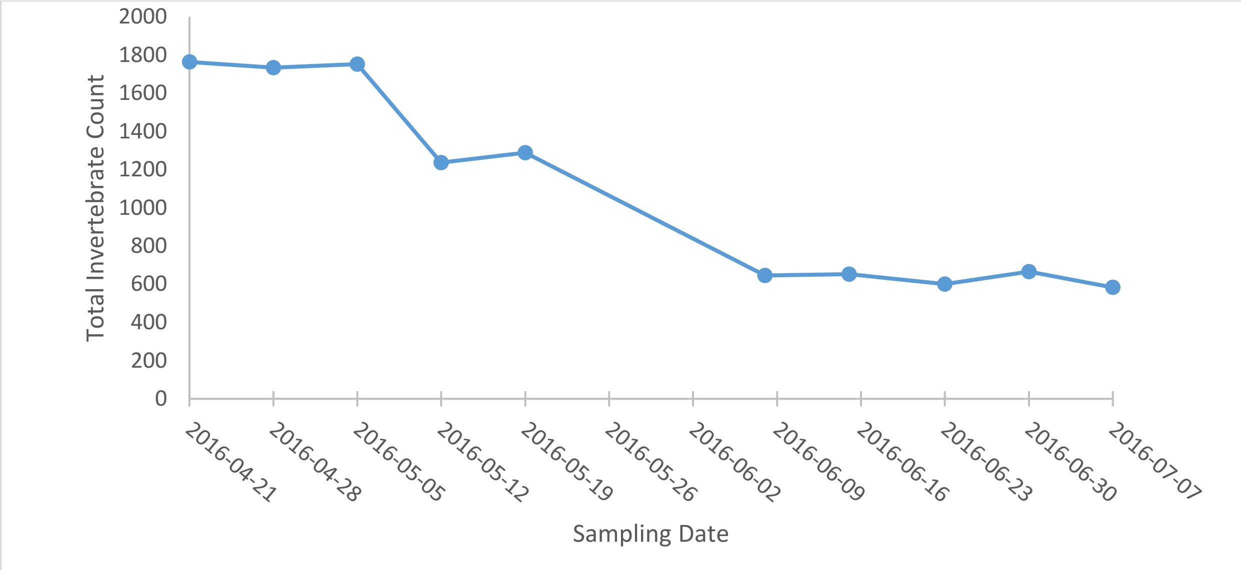

Figure 3.7 – Total invertebrate count from all 14 sites, during the invertebrate sampling period in the spring and summer of 2016, assessed by kick-net sampling with a 800 µm mesh net. Invertebrate populations declined steadily during the sampling period, starting at ~1800 in late April and falling to ~600 by July.

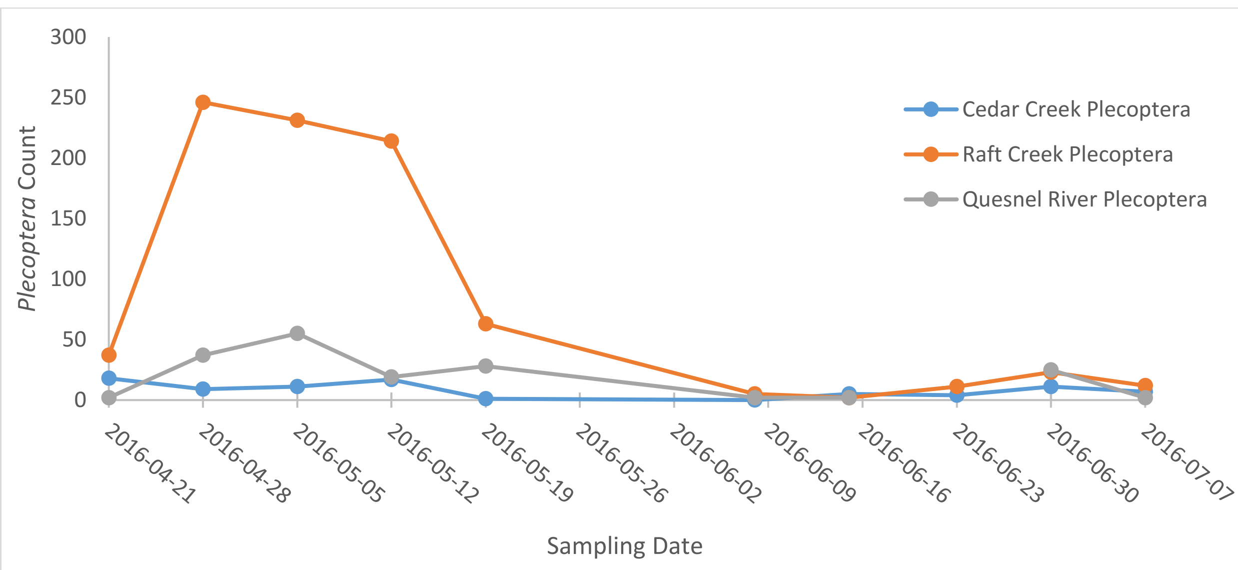

Figure 3.8 – Plecoptera (stonefly) count at three sampled creeks in the spring and summer of 2016, assessed by three one-minute kick-net samples with a 800 µm mesh net. Populations of this taxon spike in mid-May when larvae are active, before declining in June when they emerge from the aquatic environment.

3.3.3.2 Oligochaete worms and spill-associated metals

Aquatic Oligochaeta (earthworms and their relatives) are found in higher abundance at impacted sites. In potentially impacted water bodies, their counts correlate significantly with several metals associated with the Mt. Polley spill: selenium, vanadium, and copper (Table 3.1).

Table 3.1 – Correlation between Oligochaeta abundance (catch per unit effort) and concentrations of selenium, vanadium and copper in environmental samples of sediment, biofilm and collector invertebrates, with significant correlations marked with an asterisk (*).

Environmental material	Metal, correlation coefficient, and p value					
	Selenium		Vanadium		Copper	
	r	p	r	p	r	p
Sediment	0.596*	0.025*	0.822*	< 0.001*	0.357	0.210
Biofilm	0.319	0.266	0.466	0.093	0.735*	0.003*
Collector Invertebrates	0.533*	0.049*	0.021	0.942	0.134	0.647

When the abundance of Oligochaeta are compared with selenium, copper, and vanadium concentrations in environmental materials from the same sites, significant correlations are found with concentrations of copper in biofilm, vanadium in sediment, selenium in sediment, and selenium in collector invertebrates. Additionally, the count of Oligochaeta correlates positively and near-significantly with the concentration of vanadium in biofilm ($r = 0.466$, $p = 0.093$, $n = 14$).

Oligochaeta catch per unit effort correlates significantly and positively with the particle sizes of nearby sediment samples in Quesnel Lake (Table 3.2), seemingly associated with coarser sediments (in this case silts), preferring sites where the nearest sediment cores had a D_{50} greater than 10 µm. This correlation is significant for D_{10}, D_{50} and D_{90} particle size indices, but the D_{90} size fragment has the strongest relationship.

Table 3.2 – Test results from pairwise correlation of Oligochaeta abundance (catch per unit effort) in potentially impacted water bodies with three particle size indices: D_{10}, D_{50}, and D_{90} in nearby benthic sediment samples, with n = 10.

	Particle size indices					
	D_{10}		D_{50}		D_{90}	
	r	p	r	p	r	p
Correlation with Oligochaeta Abundance	0.718	0.019	0.874	< 0.001	0.905	< 0.001

Other possible factors, such as sediment nutrient content, were not found to be related to Oligochaeta abundance. The CPUE does not regress significantly with phosphorus content in nearby sediment cores ($p = 0.828$, $n = 14$). The distribution of Oligochaeta may also be spatially associated with spill impacts. In an unpaired t test, the West Basin is found to have significantly higher abundance of *Oligochaeta* than the Main Basin ($t = 2.402$, $p = 0.018$, $n = 14$), with a mean count in the West Basin of 16.4, compared to 6.0 in the Main Basin.

3.3.4 Summary of Results

This study has looked at the potential impacts from the Mt. Polley spill on sediment, biofilm and invertebrates, and has tested for biomagnification, bioaccumulation and ecological influence to impacted water bodies. To summarize the results of this research, Table 3.3 categorizes potential impacts by metal, and draws data from both Chapters 2 and 3 of this thesis.

Table 3.3 –Summary of trophic analysis, showing whether there are significant results indicating that mine activities are contributing the following trace metals, with the following effects, to the Quesnel Lake aquatic environment.

Effect	Trace metal						
	Cu	Fe	As	Se	Pb	Mn	V
Elevated in sediment ~	Yes	No	Maybe	Yes	Maybe	No	Yes
Elevated in biofilm ~	Yes	No	Maybe	No	Maybe	No	Yes
Elevated in invertebrates ~	Yes	No	No	No	Maybe	No	Yes
Bioaccumulation	Yes	No	No	Maybe	No	No	No
Biomagnification	No	No	No	Yes	No	No	No
Ecological influence	Maybe	No	No	Maybe	No	No	Maybe

Note: "Yes" indicates significant results that are believed related to mine activities. "Maybe" indicates significant results that are either contradictory, or have an ambiguous relationship to the spill. "No" indicates no significance, or no relation to the spill, based on the samples collected and analyzed to date. A tilde (~) next to an effect indicates the results of this category are largely drawn from Section 2.3, in the prior chapter.

Copper and vanadium appear to be the metals with the broadest impacts, as they are elevated in all environmental sample types, and may be exerting ecological influence. Lead and arsenic are sometimes found to be elevated in environmental samples, but do not show evidence of trophic or ecological effects. There is evidence of selenium trophic biomagnification, and also some evidence that selenium is affecting invertebrate diversity. Manganese and iron appear unrelated to Mt. Polley spill impacts.

3.4 Discussion

The Mt. Polley spill seems to be exerting a substantial influence on the metals content of many environmental samples in spill-impacted areas, particularly in the West Basin. This is reflected in the copper, vanadium, and selenium content of sediment, biofilm, and invertebrates, all of which relate spatially to the location of spilled material (Table 3.3). There is also evidence of biomagnification and bioaccumulation for selenium and copper, respectively, and evidence that selenium, copper, and vanadium have an ecological influence on benthic invertebrates.

The influence of the tailings deposit is also expressed at the base of the Quesnel Lake food web. At potentially impacted sites, there appears to be dietary transference of copper from sediment to biofilm to invertebrates, with concentrations of copper increasing at every trophic level to a maximal concentration in predatory invertebrates (Figure 3.2A). This pattern is not reflected in organisms from upstream paired sites, where all invertebrate feeding groups have similar concentrations of copper (Figure 3.2B). The results for copper appear to indicate that dietary pathways are important for this metal, and that biofilm may be acting as a vector for copper to pass from sediments into the food web. The dietary transfer of copper may also be responsible for bioaccumulation. Comparing biofilm and invertebrate feeding groups, copper increases in mean concentration such that biofilm < scrapers < collectors < predators.

However, there is no evidence that copper is biomagnifying with trophic levels, and the trophic pattern is most likely a result of bioaccumulation. Organisms are taking-up copper throughout their lifetime, through both dietary and environmental sources, but are not able to sequester and excrete this metal at the same rate. Predators are often longer-lived and larger than invertebrates at lower trophic level feeding groups (Woodward and Hildrew, 2002), and may accumulate more trace metals during a longer lifespan (Cardwell et al., 2013).

This pattern in copper contrasts with that in vanadium, which does not appear to be transferring along the food web, and which is not found at higher concentrations at higher trophic levels (Figure 3.3). Vanadium seems to be taken up by organisms from the environment, possibly from sediment to water to invertebrates, although the exact pathway is not evident from the materials sampled. The lack of a relationship between vanadium and trophic level does not indicate that the tailings deposit lacks influence on vanadium concentrations of high-trophic organisms. As discussed in the prior chapter, proximity to the source of trace metals contamination is strongly linked to vanadium concentrations in collector and predator invertebrates. Invertebrates sampled from the most highly impacted sites, Polley Lake and Quesnel Lake at Raft Creek, have anomalously high concentrations of vanadium exceeding Main Basin samples (Tables 2.5 and 2.6).

The data for selenium in Quesnel Lake shows elevated concentrations at higher trophic levels, with a strongly significant relationship between $\delta^{15}N$ derived trophic level and selenium concentration in invertebrates (excluding biofilm) in the spring ($R^2 = 0.430$, $p = 0.003$, $n = 18$), though this relationship is not found to be significant in the summer period ($p = 0.523$, $n = 15$). This selenium trophic relationship translates into a trophic magnification factor of 1.758. Selenium has the potential to biomagnify in aquatic ecosystems (Hamilton, 2004), so it is likely that these results indicate the occurrence of trophic magnification in the West Basin of Quesnel Lake, at least at the bottom of the food web, and in organisms that live near the sediment–water interface (i.e. invertebrates and biofilm). If selenium biomagnification is found to continue at higher trophic levels such as salmonids, which commonly consume invertebrates, and are commonly consumed by humans, it could represent a very serious threat to the health of this aquatic ecosystem and its users.

One notable pattern from the selenium biomagnification data is the stronger pattern between trophic level and trace metal concentrations in the spring sampling period compared to the summer. This finding mirrors some of the data from Chapter 2, in which other metal concentration patterns are found to be stronger in the spring sampling period (Figure 2.3). Two major possibilities exist to explain this seasonal difference, which are not exclusive and may be inter-related. First is the influence of inflowing creek water, which is often high in suspended and dissolved trace metals relative to concentrations in Quesnel Lake. Another possibility is that spring overturn in Quesnel Lake leads to greater mobilization of sediment-associated and dissolved metals from the benthic tailings deposit in the West Basin. As explored in the prior chapter, the signature of metals (V/Ni ratio) in biofilm from the West Basin matches is consistent with contamination from the tailings deposit, which indicates that Mt. Polley spill materials have a strong influence on metal concentrations in biota of the West Basin. This pattern also likely extends to other spill-associated metals including selenium, which is elevated in spilled tailings relative to background (MPMC, 2015a). It seems probable that the seasonal difference is, at least in part, a result of remobilizing spill material due to seasonal overturn.

The large volume of spill materials in Quesnel Lake present a selenium biomagnification risk that could affect local fisheries. Quesnel Lake is part of the Fraser River Catchment, which is critically important to British Columbia's anadromous salmon stocks (Beacham et al., 2004), and two populations of salmonids in the lake are federally listed as threatened (COSEWIC, 2012, 2016). Future research on trace metal concentrations in Quesnel Lake should seek to establish whether selenium biomagnification is occurring in salmonids. One possibility for future research is to use the heavily-impacted Polley Lake, which has large population of *Oncorhynchus mykiss* (Rainbow Trout) (Keeley et al., 2005), and is relatively self-contained such that fish there have difficulty migrating to less-impacted habitat. A study using stable isotopes to quantify trophic levels in this system, in conjunction with metals data from organisms, may find further evidence of selenium trophic magnification.

A study on the effects of coal mining on selenium concentrations in Cutthroat Trout (*Oncorhynchus clarkii*) in the Elk River Watershed of southeastern British Columbia, found that selenium concentration in eggs related significantly with fertilization success and alevin mortality (Rudolph et al., 2008). In this same study, however, Rudolph et al. (2008) suggest that the no-effect threshold for this relationship between selenium and deformities is 20.6 mg/kg in egg tissues, above which selenium concentrations in eggs were significantly related to negative health outcomes. This study did not find selenium concentrations in any organism of the Quesnel Catchment to exceed 6 mg/kg, but with a trophic magnification factor of 1.756, it would take approximately two additional trophic levels to approach the 20.6 mg/kg threshold. Additionally, the samples in which selenium biomagnification was detected were taken approximately 20 months after the spill event in 2014. In lake environments, selenium cycles continuously in the food web and it can take many years for trace metals to reach steady-state concentrations (Brandt et al., 2017). The fish of Quesnel and Polley Lakes would benefit from long-term monitoring to establish with some certainty whether selenium magnification is occurring.

The concentration of selenium and other metals in Quesnel Lake appears to also have ecological consequences for invertebrates. Aquatic oligochaete worms (subclass Oligochaeta) are found in higher abundance in Quesnel Lake at sites where metal data indicates tailings impacts. Their abundance correlates significantly with selenium concentration in nearby sediment ($r = 0.596$, $p = 0.025$, $n = 14$), selenium concentration in collectors ($r = 0.533$, $p = 0.049$, $n = 14$), copper concentration in biofilm ($r = 0.735$, $p = 0.003$, $n = 14$), and vanadium concentration in nearby sediment ($r = 0.822$, $p < 0.001$, $n = 14$). Collectively these indices point to the possibility that Oligochaeta are tolerant of environments that are more heavily impacted by Mt. Polley spill materials, or that Oligochaeta are acting as colonizers in the spill-impacted West Basin. One aspect of the spill impact that appears to be influential is particle size. Oligochaete worms are found to be significantly more abundant at sites with larger median particle size (D_{50}) in nearby sediment cores ($r = 0.874$, $p < 0.001$, $n = 10$). Other studies examining newly exposed substrata find that Oligochaeta often act as colonizer species. In a study of colonization of artificial sedimentary substrata in a river in Japan, Olomukoro and Tochukwu (2006) found that Oligochaeta were among the first invertebrates to colonize. In another examination of colonization of sediments near macrophyte roots in Brazil, de Lima Behrend et al. (2013) noted that “most of Oligochaeta… are opportunistic colonizer[s]”. It appears that oligochaete worms are colonizing tailings-impacted environments (particularly those with larger particle sizes), and that these worms are more tolerant of elevated trace metal concentrations than other invertebrate taxa examined in this study. As some Oligochaeta species, particularly T*ubifex ssp.*, are commonly used as environmental indicators for trace metals (Bouché et al., 2000; Rathore and Khangarot, 2003; van der Geest and Paumen, 2008), this relationship may be worthy of a more targeted follow-up study in which oligochaetes are better identified, and specifically tested for their trace metal concentration.

The colonization of Oligochaeta in spill-impacted environments is a finding from this invertebrate study which may reflect the future of Quesnel Lake benthic invertebrates. It appears that spill-impacted benthic environments have a distinct invertebrate community, reflective of changes to the physical and chemical environment. The spill deposit, which buried native aquatic benthic habitat (MPMC, 2015b), is being recolonized in what could be seen as a sign of recovery, and of resilience in the aquatic ecosystem. Other studies have found signs of benthic recolonization in both Quesnel and Polley Lakes (Golder, 2018). However, the recolonizing benthic invertebrate communities do not represent a recovery of the pre-spill environment, but the growth of a new and distinct invertebrate community that is better-adapted to the characteristics of the benthic environment of the post-spill West Basin. The spill has created a novel ecological niche which is in the process of being colonized. Oligochaeta may be a perfect exemplar of the new community. They appear to be tolerant of higher concentration of trace metals, particularly selenium, copper, and vanadium. They also seem to be adapted to the physical characteristics of the spill deposit, such as particle size and possibly low carbon content. To understand the resiliency, recovery, and ecological trajectory of Quesnel Lake, it is worthwhile to monitor the recovery of benthic macroinvertebrates in the West Basin.

3.5 CONCLUSION

This chapter sought to explore the trophic transfers of trace metals in water bodies impacted by the Mt. Polley Mine spill, in comparison to nearby unimpacted water bodies. Specifically, it was hypothesized that spill-associated metals including copper and vanadium would be bioaccumulating in impacted water bodies, and that selenium may be biomagnifying. Additionally, biofilm was hypothesized to be a vector for trace metals to pass from the abiotic environment into the food web. Additionally it was hypothesized that invertebrate abundance would be related to spill impacts in the form of trace metal concentrations.

Quesnel Lake food web analysis indicates selenium biomagnification was occurring in aquatic invertebrates during the 2016 spring sampling period, with a magnification factor of 1.758. Copper was found to be bioaccumulating and found in the highest concentrations at high trophic levels. Food web transfer for copper was detected among most links in the food web at impacted sites, and there was evidence of biofilm acting as a vector between sedimentary copper and the invertebrate food web. The aquatic invertebrate taxa Oligochaeta was found to be more abundant in spill impacted environments and was also found to be associated with elevated concentrations of copper, selenium, and vanadium in environmental samples. Collectively these results indicate that the Mt. Polley spill is impacting trace metal cycling at the base of the food web and pose an ecological risk to the aquatic environment of Quesnel Lake.

4 CONCLUSION

4.1 RESEARCH SUMMARY

The Mt. Polley Mine spill of 2014 provides a unique opportunity to study a catastrophic environmental event. The large volume (~25 M m^3) of material released into the landscape (Petticrew et al., 2015) makes it one of the largest mining spills in Canadian history (Sussbauer, 2017). This study sought to identify patterns of metals associated with the spill in environmental samples of water, biofilm, and invertebrates, and to evaluate how these elements are affecting the base of the Quesnel Lake food web. It looked at whether biofilm may be a vector for abiotic metals to enter the food web, sought evidence of bioaccumulation or trophic biomagnification, and to assess the relative importance of dietary and environmental exposure for metal uptake. It also sought to establish the relative importance of natural and anthropogenic trace metals inputs to Quesnel Lake, and distinguish between discharge via pipe diffuser and tailings associated with the spill from the Mt. Polley Mine. All of these objectives have been fulfilled to some degree. This conclusion chapter summarizes results which are found elsewhere in this document.

4.2 SPATIAL PATTERNS OF TRACE METALS IN QUESNEL LAKE

This study documents many significant spatial relationships when analyzing trace metal concentrations in different areas of Quesnel Lake, some of which are believed to be related to the Mt. Polley spill. Metal concentrations were analyzed in sediment, water, biofilm, and invertebrates. Analyses fall broadly into two categories: binary comparison of the West Basin and Main Basin of Quesnel Lake, and continuous analysis along a distance variable, defined as distance from where spill materials initially entered Quesnel Lake (Appendix A).

The highest concentrations of copper and vanadium are found in locations impacted by the Mt. Polley spill. For copper, this pattern holds true for every sample type analyzed (sediment, water, biofilm, all invertebrate feeding groups), and when analyzed both by continuous and binary methods. Copper concentrations are highest overtop the tailings deposit in the West Basin. For an example of how pronounced this pattern is, mean biofilm, invertebrate, and DGT values for copper in Quesnel Lake are all found to be maximal at the Raft site, approximately 1 km from where spill materials entered the lake. The spatial pattern of vanadium is somewhat less pronounced than that of copper but shows the same overall pattern with elevated concentrations in the West Basin in biofilm, invertebrates, and sediment. Vanadium is also found to be useful as a tracer of spill-related materials in conjunction with nickel (i.e. the V/Ni ratio) and is able to specifically identify spill-associated metals from the background levels in the environment. The V/Ni ratio is associated spatially with the spill deposit in biofilm samples (Figure 2.6).

Other trace metals, including selenium, arsenic, and lead, show some spatial patterns, but these patterns have an uncertain relationship with the tailings deposit, and further research is required to understand the cause of such patterns. Arsenic is elevated in the West Basin but is highest to the west of the main tailings spill deposit (Figure 2.5), indicating that another source may be primarily responsible for the high readings, and may be associated with fluvial inputs from local creeks. Similarly, lead shows a high concentration in biofilms sampled from the East Arm (Figure 2.5), and it is not clear whether lead is associated spatially with the tailings deposit, though in some invertebrate feeding groups, lead concentration does seem to be highest near the spill site (Tables 2.5 and 2.6). Selenium, a metal enriched in tailings, is sometimes found in high concentrations near the tailings deposit, but this pattern is not consistent. Polley Lake samples of invertebrates and sediment are elevated in selenium, but samples of biofilm are not found to be different between the West and Main Basins (Figure 2.3). Although the spatial pattern of selenium is unclear, there is evidence of selenium trophic biomagnification in impacted environments, which is elaborated upon further below.

4.3 Trophic Patterns of Trace Metals in Quesnel and Polley Lakes

Trophic analyses targeted copper, vanadium, and selenium as metals of potential interest to the biota in areas impacted by the Mt. Polley spill. Analysis of trophic patterns utilize two techniques: a simplified food web to examine the correlation and transference of metal between materials in the environment, and stable isotope ratios which are used to quantify trophic positioning, with these quantified trophic levels used to assess biomagnification. These techniques are used to seek evidence of bioaccumulation and biomagnification, to see whether biofilm is functioning as a vector for trace metals into biota, and to determine the relative importance of dietary and environmental exposure of trace metals on organisms.

Copper does not appear to be biomagnifying in the aquatic food web downstream from the Mt. Polley spill, but there is some evidence of bioaccumulation, with organism copper concentrations generally highest in the predator invertebrate feeding group (Figure 3.2). Based on the correlation and transference factors in the food web of potentially impacted sites, it appears that dietary transfer of copper is substantial, and that biofilm does act as a vector for copper in sediment to enter the food web. The same is not true for vanadium in the food web, which does not display a pattern of bioaccumulation (Figure 3.3), and which appears to be adsorbed into the food web primarily through environmental exposure, not through dietary uptake.

Selenium behaves differently in the aquatic environment and does show signs of trophic biomagnification in Quesnel and Polley Lakes during the spring sampling period. Nitrogen stable isotope ratios, used to quantify trophic levels, show a clear pattern of increasing selenium concentration with increasing trophic level (Figure 3.5), with a trophic magnification factor of 1.76. Selenium appears to transfer from sediment to water to organisms, with strong significant correlations between waterborne DGT-labile selenium and organism body loads in biofilm and collector invertebrates.

4.4 Distinguishing Tailings Spill Contaminants from Wastewater Discharge

Quesnel Lake has two separate sources of potential pollution, both related to the Mt. Polley Mine. First is the tailings deposit in the West Basin, which has contributed metals to the lake's water column since 2014. Second is the ongoing discharge of mine operation wastewater into the West Basin hypolimnion, which has taken place since 2015. The tailings deposit itself spans approximately 2.75 km^2 on the Quesnel Lake benthos, with a much larger area impacted by halo effects. Copper concentrations (Figure 2.5) and V/Ni ratios (Figure 2.6) in biofilm indicate that Polley Lake, Quesnel Lake's West Basin, and the upper reaches of the Quesnel River are affected, and that the impact diminishes over the scale of tens of kilometers. Some spill effects tested in this study, including binary spatial analysis and selenium trophic biomagnification, appear to be greatest in magnitude during the spring sampling period. As the spring sampling period is designed to capture conditions during lake overturn, it is hypothesized that vertical mobilization of the spill deposit could explain this difference. Both the large spatial scale affected by metal contamination, and the seasonal change, indicate that the tailings deposit is more impactful than wastewater discharge. It should be noted that the ability to distinguish between anthropogenic impacts is a recognized deficiency with this biomonitoring study design. Targeted research should investigate this further.

4.5 The Influence of Anthropogenic and Natural Sources of Trace Metals

Historic mining activities in the landscape surrounding Quesnel Lake, and the placement of a large open-pit mine at the Mt. Polley site, make it apparent that metal-rich mineral deposits are a natural feature in the area. With the data presented here, it would not be possible to assess quantitatively the relative importance of natural and anthropogenic sources of bioavailable trace metals, but the data does contain some patterns that are more indicative of anthropogenic mine impacts. In samples from this study, metal concentrations in biofilm from inflowing tributary creeks correspond with, and sometimes exceeded, those from spill-impacted environments in Quesnel Lake, making it obvious those creeks are delivering metals to Quesnel Lake (Appendix C). However, the metal taken up by biofilm from inflowing creeks is different from that in Quesnel Lake. Elevated values of copper and vanadium in tributary environments are found to be related to sediment entrainment by biofilm, indicating that inflowing tributary creeks, which are actively weathering and eroding the landscape, are delivering metals primarily in particulate form (Appendix C). Additionally, the inflowing creeks contain a very small volume of water relative to Quesnel Lake, so metal load inputs to the lake are likely to be low relative to the loading associated with the Mt. Polley spill and other mine-related activities.

Copper concentrations and V/Ni ratios in sediment, biofilm, and invertebrates are another indicator of the relative influence of natural and anthropogenic bioavailable metal sources. The

V/Ni ratio in environmental samples increases by approximately double between the West and Main Basins of Quesnel Lake. At sites in Polley Lake and near the Hazeltine Creek mouth, V/Ni ratios are approximately three to four times as high as those in the Main Basin (Figure 3.6). Copper indicates a broadly similar spatial pattern, with concentrations increasing from the Main Basin < West Basin < Polley Lake over the scale of tens of kilometers (Figure 3.5). Additionally, there is no indication of copper bioaccumulation or selenium biomagnification in upstream tributary streams, while this does appear to be occurring at impacted sites in Polley and Quesnel Lakes (Figure 3.2). The simplest explanation for these findings is that the tailings spill has disrupted a previously equilibrated system, causing organisms to be exposed to more bioavailable trace metal than they would have been, had the spill not occurred.

4.6 Ecological Implications

The aquatic environment of Quesnel Lake is regionally important for many reasons. It is the source of drinking water for the nearby town of Likely, it is a popular recreation destination within British Columbia, it contains habitat for protected fisheries, and has cultural importance to T'exelcemc and Xatśūll First Nations communities. The mine spill, and the increase in bioavailable trace metal, have several potential implications to ecological management, mostly relating to fisheries.

Although water quality in Quesnel Lake presents a risk to aquatic life, the findings from this study do not indicate that conditions are acutely dangerous to humans at present. Dissolved trace metal content in the water (measured by DGT) is well below human safety standards for bathing and drinking (Health Canada, 2017). Suspended sediment in drinking water could potentially pose a risk to humans upon ingestion, and CTD measurements from the Quesnel

River Research Centre show periods of elevated turbidity in the hypolimnion of the West Basin site (E. Petticrew, unpublished data, 2015), relative to the Main Basin site. Another potential risk of exposure to humans is the regular consumption of fish caught from Quesnel Lake. If the trends of biomagnification and bioaccumulation continue to fish, it could be possible for humans to ingest trace metals in excess of safety guidelines. However, this study did not investigate metal concentrations in fish tissues, nor drinking water uptake, so it would take further study to substantiate these possibilities.

An interesting outcome from the Mt. Polley tailings spill is that its aquatic impact could have been much worse had geochemical conditions allowed for acid mine drainage (AMD) to occur on a large scale. AMD would have caused additional leaching of metals from spilled materials (Akcil and Koldas, 2006), and allowed for a substantial release of dissolved, bioavailable trace metals that could have impacted a larger portion of the Fraser River system. As it happened, Quesnel Lake was spared this effect primarily because of high calcite and low sulfur concentrations in the ore body being mined. This incident is an important reminder that the buffering capacity of water bodies, and the AMD potential of mine waste, is an important factor in determining the severity of possible aquatic impacts.

Looking into measurable ecological changes in the Quesnel Lake region, taxonomic identification of invertebrates in this study revealed that Oligochaeta (segmented worms or sludge worms) are found in higher abundance at sites with higher concentration of vanadium, selenium, or copper in associated environmental samples (Section 3.3.3). It seems likely this is due to some affinity for the post-spill environment in Quesnel Lake. This may be a tolerance of the trace metals in spill materials, or a preference for larger particle sizes, or simply colonization of a disturbed environment by opportunistic species. This colonization can be interpreted as recovery of the benthic ecosystem in the West Basin, but after this major disturbance event, the environment is different, and the ecological community will reflect this. Specifically, the surface of the tailings deposit has lower cohesion, less organic matter, higher trace metals, and coarser particle sizes than the surrounding benthic sediment (E. Petticrew, unpublished data, 2016), and the recovery of the environment will favor species with a preference or tolerance for these characteristics.

The Quesnel Lake ecosystem has demonstrated some degree of resiliency, both chemical and ecological, following the Mt Polley Mine spill, but the time period associated with a full recovery is uncertain, if at all possible, in that a new state of equilibrium may be reached. The tailings spill deposit will forever be present as a unique feature in the lake bed's depositional chronology, and until the layer is fully buried by fresh deposition (possibly in a time period of a few decades to a few centuries), tailings may continue to exert a chemical influence on the surrounding water body. For this reason, one recommendation of the present work is that ongoing monitoring and sampling are required to assess the response of this aquatic ecosystem to a major environmental disturbance event.

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Appendixes

A. Measuring Distance in Quesnel Lake

Distances in Quesnel Lake and the Quesnel River were measured using distance paths in Google Earth (Figures A2, A3). Paths were drawn in a branching pattern starting at the mouth of Hazeltine Creek in Quesnel Lake. This path follows the thalweg of Quesnel Lake (the deepest central channel) and bifurcates to access sampling locations near the shores. These distances are meant to represent a path that spill materials could have travelled to reach different areas of the lake. Distances were measured to sites in Quesnel Lake and the Quesnel River. The Polley Lake site had its distance set to 0 km, representing an environment highly impacted by tailings and other spill material. Distances were not measured to inflowing tributary creeks, which are upstream of spill materials.

Figure A1 – Tile sampling locations in Western Quesnel Lake.

Figure A2 – Distance Paths to sites in the western portion of Quesnel Lake, including tile sites shown in A1 and buoy sites shown in A3.

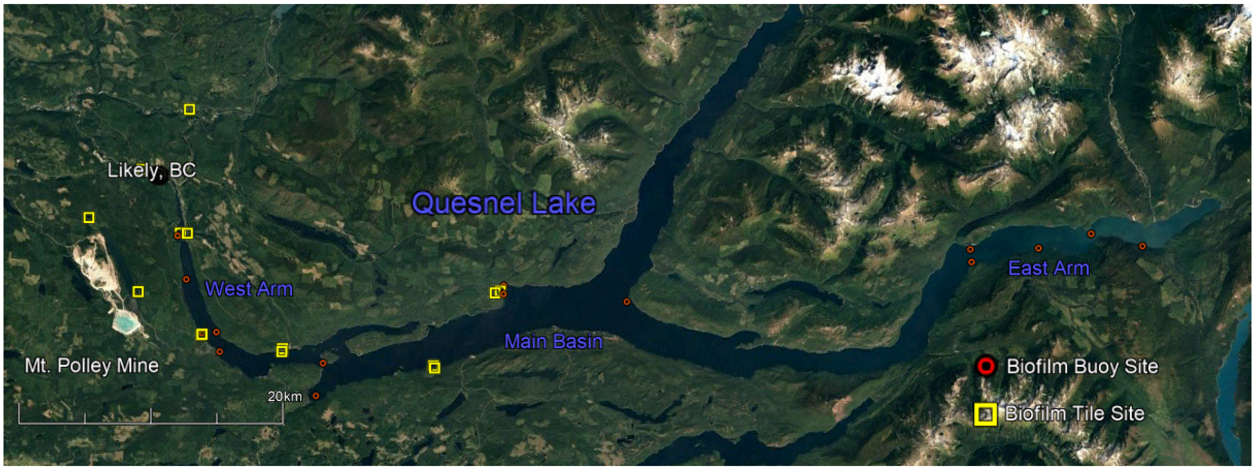

Figure A3 – All sampling sites in Quesnel Lake and surrounding water bodies.

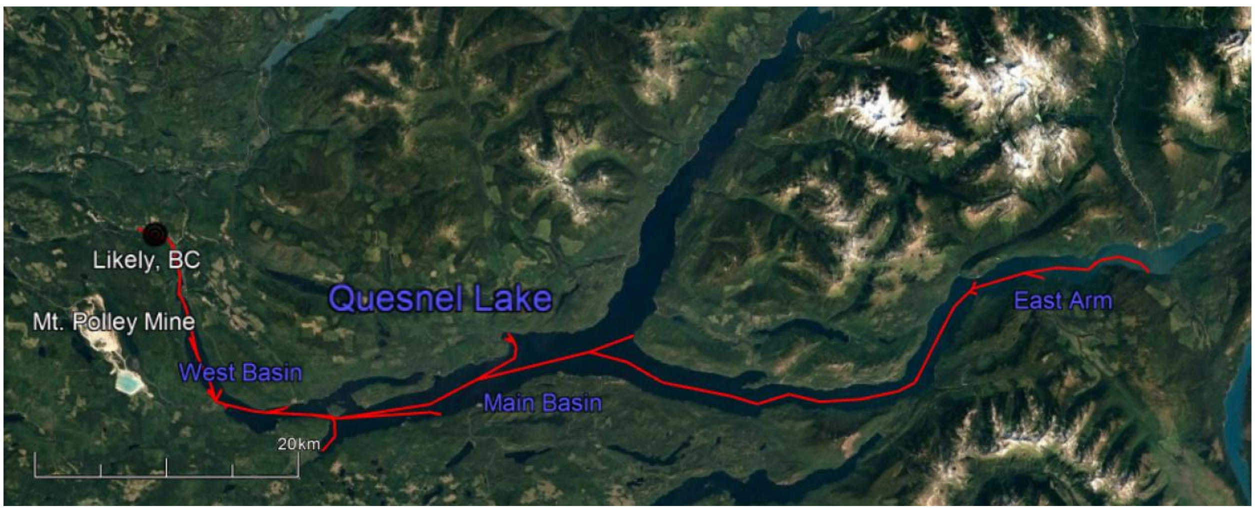

Figure A4 – Distance paths to all sampling sites in Quesnel Lake and the Quesnel River

B. Metals Associations in Biofilms

Looking at trace metals' correlations in sampled biofilm from the Quesnel Catchment, distinct patterns emerge depending on which types of samples are analyzed. Tile-grown biofilm sites within Quesnel Lake have a distinct pattern of trace metal concentrations, as these are the ones most directly impacted by the Mt. Polley spill (Table B1). Another distinct pattern is observed at tile sampling sites that are upstream from the spill impacts, in tributary environments

(Table B2). And a third distinct pattern is observed in buoy-grown biofilms spanning the entirety of Quesnel Lake (Table B3).

Table B1– Correlation coefficients of 10 metal concentrations in biofilm grown on artificial tile substrates in potentially impacted areas: Quesnel Lake, Polley Lake, and the Quesnel River. Near-significant results with $p < 0.1$ are marked with a single asterisk (*), while significant results with $p < 0.05$ are marked with double asterisks (**). Meaningful correlations are shaded blue if positive, and red if negative.

	V	Cu	Pb	Al	As	Mn	Cd	Se	Ni	Cr
V	1									
Cu	0.819**	1								
Pb	0.572**	0.390	1							
Al	0.687**	0.470*	0.635**	1						
As	0.498*	0.455	0.506*	0.331	1					
Mn	-0.681**	-0.599**	-0.549**	-0.694**	-0.502*	1				
Cd	-0.612**	-0.443	-0.532*	-0.773**	-0.348	0.709**	1			
Se	-0.158	0.020	-0.007	-0.226	-0.347	0.050	0.0061	1		
Ni	0.454	0.164	0.559**	0.826	0.531*	-0.583**	-0.574	-0.022	1	
Cr	-0.039	-0.147	0.067	0.567**	-0.123	0.036	-0.356	0.495*	0.492*	1

At impacted tile sites, representing western Quesnel Lake, two groups of metals are evident which have intra-group positive correlations, and inter-group negative correlations. The first group is copper, vanadium, lead, aluminum, and arsenic. All permutations within this group have a correlation coefficient of at least 0.3, and all correlations are significant ($p < 0.05$) or near significant ($p < 0.1$), except for lead-copper, arsenic-copper, and arsenic-aluminum (Table B1). The second group is manganese and cadmium, which correlate strongly and significantly ($p = 0.004$, $r = 0.708$) with each other, but negatively and significantly with metals in group 1, except for cadmium-copper and cadmium-arsenic, whose correlations are negative but not significant. Selenium, despite being elevated in spill materials (MPMC, 2015a), does not appear to be correlated with other metals in western Quesnel Lake (Table B1), including ones that are elevated in spill materials.

Table B2 –Correlation coefficients of metal concentrations in biofilm grown on artificial substrates in environments upstream from, and unimpacted by, the Mt. Polley spill. Near-significant results with $p < 0.1$ are marked with a single asterisk (*), while significant results with $p < .05$ are marked with double asterisks (**). Meaningful correlations are shaded blue if positive, and red if negative.

	V	Cu	Pb	Al	As	Mn	Cd	Se	Ni	Cr
V	1									
Cu	0.614**	1								
Pb	-0.087	-0.129	1							
Al	0.681**	-0.001	0.203	1						
As	0.125	0.406	0.288	-0.073	1					
Mn	0.377	-0.094	0.113	-0.654**	-0.047	1				
Cd	-0.195	-0.185	0.884**	0.105	0.414	0.309	1			
Se	-0.205	-0.345	0.907**	0.100	0.134	0.242	0.982**	1		
Ni	0.819**	0.400	0.316	0.617**	0.261	0.507*	0.286	0.262	1	
Cr	0.758**	0.179	-0.026	0.603**	-0.024	0.670**	0.057	0.104	0.835**	1

At unimpacted sites which are upstream from the Mt. Polley Mine spill, metals in biofilm have different patterns, with many more significantly positive than negative correlations among all metals. Comparing potentially impacted (Table B1) and unimpacted (Table B2) tile sites, the only significant correlations which are consistent are copper-vanadium, aluminum-vanadium, and manganese-aluminum. At unimpacted sites, the correlation patterns from group 1 (copper, vanadium, lead, aluminum, arsenic) are largely absent, and a third group of metals is evident which is not present at potentially impacted sites. This is selenium, lead, and cadmium, which are very strongly and significantly correlated at upstream unimpacted sites, with $r > 0.85$ and $p < 0.001$ for all permutations between the three (Table B2).

Table B3 –Correlation coefficients of 10 metal concentrations in biofilm recovered from buoys across Quesnel Lake. Near-significant results with p < 0.1 are marked with a single asterisk (*), while significant results with p < .05 are marked with double asterisks (**). Meaningful correlations are shaded blue if positive, and red if negative.

V	1									
Cu	0.749**	1								
Pb	-0.336	-0.517*	1							
Al	-0.261	-0.299	0.823**	1						
As	0.315	0.558**	-0.220	0.130	1					
Mn	0.423	0.473*	-0.597**	-0.302	0.722**	1				
Cd	0.404	0.221	-0.350	-0.434	0.394	0.669*	1			
Se	-0.041	0.266	-0.397	-0.029	0.833**	0.779*	0.446	1		
Ni	-0.055	-0.124	0.445	0.613*	0.298	-0.116	0.024	0.176	1	
Cr	0.508*	0.544**	0.015	0.323	0.708**	0.479*	0.336	0.472*	0.382	1
	V	Cu	Pb	Al	As	Mn	Cd	Se	Ni	Cr

Buoy-grown biofilms (Table B3), representing all of Quesnel Lake from the West Arm to the East Arm correlate in a different pattern compared to the tile-grown biofilms from western Quesnel Lake (Table B1). A vestige of the Group 1 metals (copper, lead, aluminum, arsenic) from Table 3.2 is still evident with buoy-grown biofilms, with significant correlations among copper-vanadium, copper-arsenic, and aluminum-lead. However, other intra-group metal correlations from group 1 are not significant, and copper-lead is significantly negatively correlated. Another important pattern from buoy-grown biofilms is that chromium correlates significantly or near-significantly with vanadium ($r = 0.508$), copper ($r = 0.544$), arsenic ($r = 0.708$), manganese ($r = 0.479$) and selenium ($r = 0.472$). This is an interesting detail because chromium and nickel are known to be lower in spilled mine tailings than in background sediments from Quesnel Lake (E. Petticrew, unpublished data, 2016). Manganese is another metal with major differences in patterns between buoy-grown and tile-grown biofilm samples in Quesnel Lake. In tile-grown samples (Table B1), manganese correlates significantly and negatively with vanadium, copper, lead, aluminum, arsenic, and nickel. However, in buoy-grown biofilms the only one of these negative correlations to remain is between lead and manganese.

Also different from the tile-grown biofilm results is that manganese is positively correlated with copper and arsenic (Table B3).

Further analysis is required to understand how these patterns of trace metal concentrations in biofilm relate to Mt. Polley Mine spill materials. The association of copper, lead, vanadium, and arsenic concentration in tile-grown biofilms may be an identifiable signal of spill impacts. A vestige of this pattern can be identified in the buoy-grown biofilms that span the entirely of Quesnel Lake, but the metal groups are not present in upstream paired sites. It seems likely that group 1 metals (copper, vanadium, lead, aluminum, arsenic; Table B1) are associated with the tailings spill.

C. Paired Biofilm Sites and Naturally Inflowing Metals

Natural variations exist in the geochemistry of Quesnel Lake, and inflowing creeks may be contributing metals to the lake, independent of mine operations. When the metal contents of biofilms from upstream and downstream site pairs are compared, it appears that streams draining into the West Basin are naturally elevated in copper, and to a lesser extent, arsenic and vanadium, in a pattern matching the metal concentrations at the Quesnel Lake sites (Figure C1).

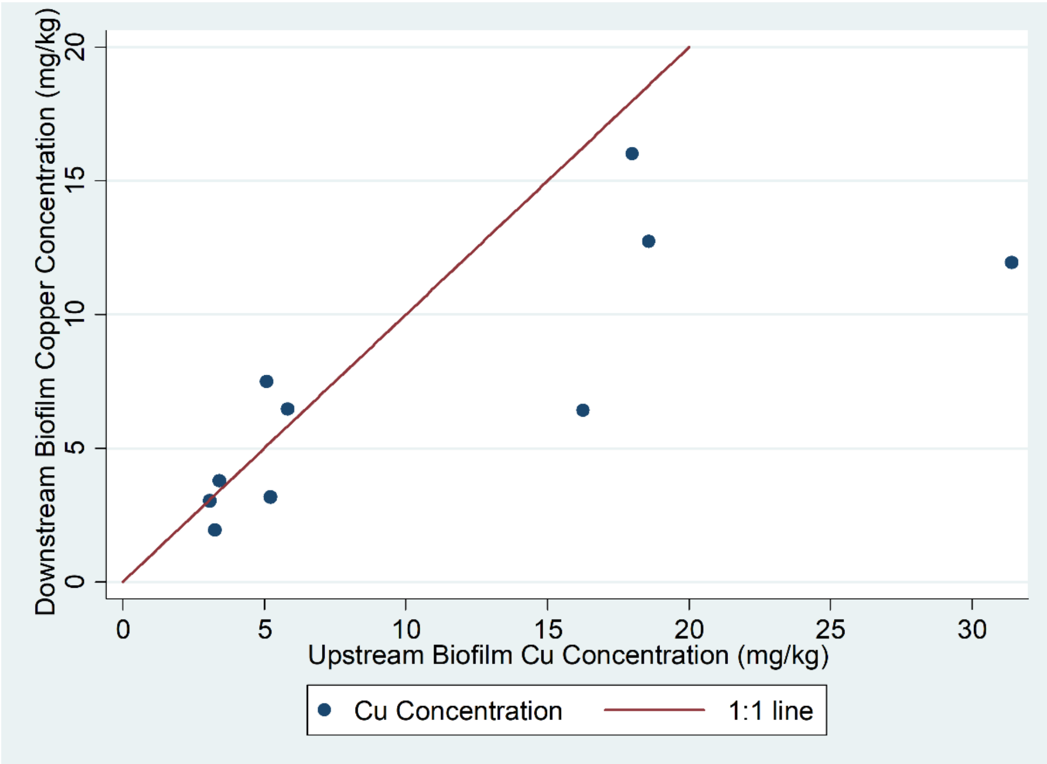

Figure C1 – Copper concentrations in biofilm from paired sites pairs in Quesnel Lake (downstream) and its tributaries (upstream), with a y = x line for reference. Some values correspond between site pairs, but where values fall below the line, upstream copper concentrations in biofilm are higher than at the paired downstream sites.

This pattern for copper makes it appear that the natural metal loads of inflowing creeks may be partly responsible for the elevated copper content in the West Basin, indicating the metal patterns in Quesnel Lake are not solely attributable to the mine spill and ongoing discharge. However it should be noted that even at their peak flow, the sampled creeks which are inflowing to Quesnel Lake have very small discharge (on the order of 1 m^3 / s), relative to the volume of Quesnel Lake (43 km^3).

As shown above (Figure C1), in some cases there is correlation between biofilm metal concentrations at the paired sites. However, there are important differences between the metal concentrations at upstream and downstream sites, which are explored here by examining the difference between downstream and upstream site pairs.

$$\Delta[\text{analyte}] = \text{downstream}[\text{analyte}] - \text{upstream}[\text{analyte}]$$

Equation C1

The change in analyte concentration between the downstream and upstream site is referred to as Δ[analyte] (Equation C1), where analyte in this case is the metal concentration in biofilm samples from different environments. A negative Δ value indicates that the concentration of metal is lower in the downstream (Quesnel Lake) site while a positive Δ value indicates lower metal content in the upstream (tributary stream) site.

Tile sample sites in Quesnel Lake utilized moorings with biofilm substrate tiles in the photic zone, while sites in other water bodies had tiles attached directly to anchors placed on the streambed. This was done as an adaptation to the differing sizes of water bodies sampled. However, it was discovered that biofilms grown on tile moorings in Quesnel Lake collected significantly less sediment than biofilm grown on anchors in streambeds, making the upstream and downstream site pair comparison imperfect. The mean sediment content of biofilms from Quesnel Lake moorings is 2.69% (St. dev. 3.54), compared to 12.7% (St. dev 8.34) at upstream sites. This difference is significant by unpaired t-testing ($t = 3.085$, $p = 0.007$, $n = 10$). The following analysis aims to assess the influence of sediment content, by comparing the five sites in Quesnel Lake (where moorings were used) with five upstream paired sites (where streambed anchors were used). The upstream sites are in: Whiffle Creek, Abbott Creek, Winkley Creek, Cedar Creek, and Raft Creek. The downstream sites are in Quesnel Lake, near the bays where the above streams enter the lake.

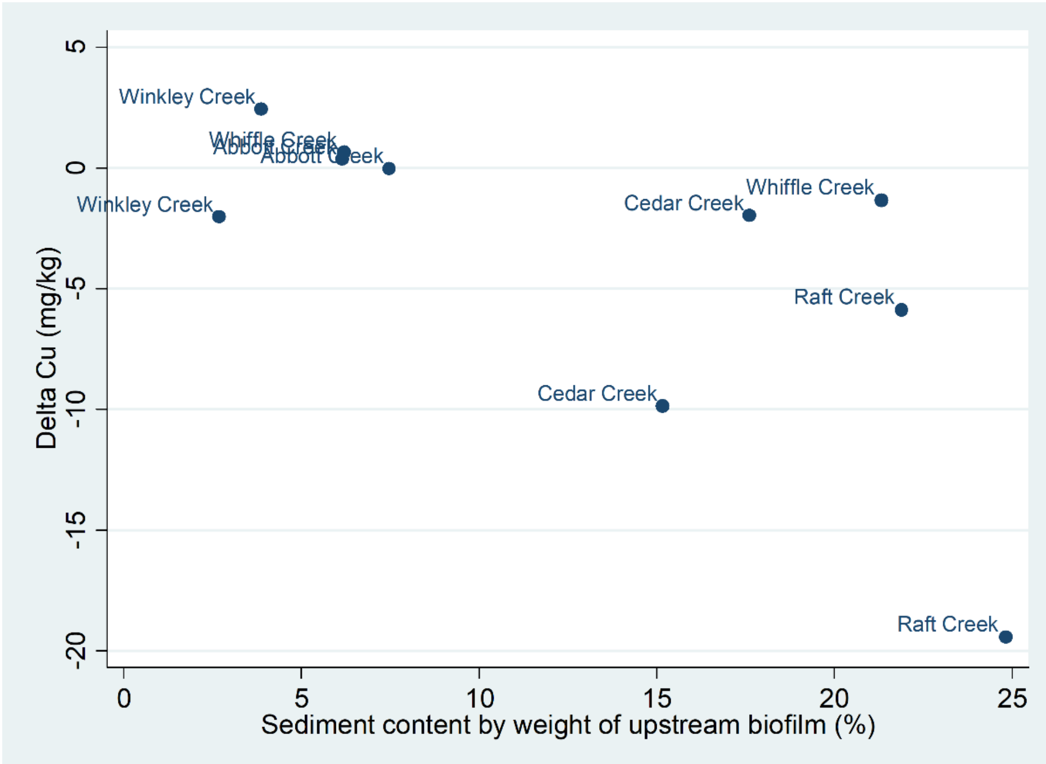

Figure C2 – Change in copper concentration between upstream and downstream site pairs (ΔCu), plotted against the sediment content in the upstream biofilm. Both seasons are represented in this figure, so each site name is repeated.

In the plot showing the relationship between ΔCu and the sediment content of the upstream biofilm (Figure C2), it is evident that a high sediment content (above 10% by weight) is associated with a negative ΔCu value. This relationship correlates significantly ($r = -0.693$, $p = 0.026$, $n = 10$), indicating that the biofilm copper contents at upstream sites are inflated because of sediment that becomes entrained in biofilm in these high-energy environments.

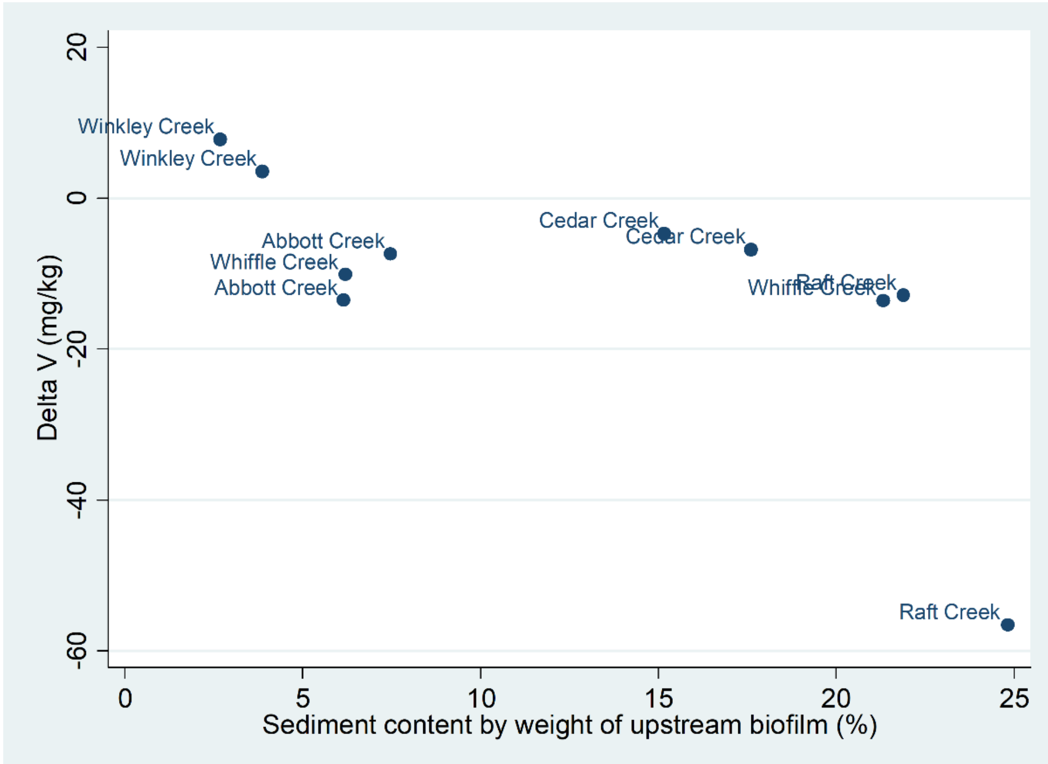

Figure C3 - Change in vanadium concentration between upstream and downstream site pairs (ΔV), plotted against the sediment content in the upstream biofilm. Both seasons are represented in this figure, so each site name is repeated.

The relationship between ΔV and the sediment content at upstream sites shows that low and negative ΔV values are associated with high sediment content in upstream biofilm samples. This correlation is significant ($r = -0.673$, $p = 0.033$, $n = 10$), indicating that elevated values at upstream sites are inflated by their sediment content. This effect from sediment is important, because biofilm metal content is meant to represent the uptake of bioavailable metal in the aquatic environment, and sediment entrainment does not properly represent this uptake.

D. Diffusive Gradients in Thin-Films Data

To assess concentrations of trace metals in aqueous solution, diffusive gradients in thin-films (DGT) methodology was used. These data are used in food web diagrams (Figures 3.2 – 3.4), but the DGT results are not reported elsewhere. These data represent dissolved, ionic trace metals sampled *in situ*, as explained in Section 1.6. Data are separated by sampling period and values are presented in micrograms per litre.

Table D1 – Diffusive gradients in thin-films (DGT) concentrations of dissolved, ionic trace metals in water bodies of the Quesnel Basin during the spring sampling period, May 4 - 11, 2016. Site pairs are listed together and have the same shading. Samples below detection limit are listed "DBL".

	Spring DGT Trace Metal Concentrations (μ/L)						
Site	Cu	Fe	As	Se	Pb	Mn	V
Quesnel Lake at Abbott Creek	0.300	0.052	0.027	BDL	BDL	0.729	0.051
Abbott Creek	0.596	12.8	0.016	DBL	0.002	1.26	0.043
Quesnel Lake at Whiffle Creek	0.302	1.83	0.054	BDL	BDL	1.61	0.102
Whiffle Creek	5.77	11.8	0.069	0.002	0.003	2.53	0.927
Quesnel Lake at Winkley Creek	0.601	9.77	0.040	BDL	0.006	1.05	0.071
Winkley Creek	0.522	3.66	0.072	0.003	0.007	1.01	1.01
Polley Lake	0.384	0.733	0.198	0.031	BDL	1.37	0.465
Frypan Lake	1.68	2.27	0.009	DBL	0.004	1.33	0.557
Quesnel Lake at Raft Creek	1.90	0.022	0.045	BDL	BDL	0.676	0.081
Raft Creek	0.900	1.30	0.083	DBL	0.002	0.284	0.963
Quesnel Lake at Cedar Creek	0.136	1.18	0.080	BDL	0.002	0.268	0.095
Cedar Creek	0.730	7.89	0.291	0.004	DBL	0.643	0.197
Quesnel River	0.142	8.83	0.117	0.008	0.137	25.1	0.150
Cariboo River	1.68	7.59	0.052	DBL	0.006	1.35	0.014

Table D2 – Diffusive gradients in thin-films (DGT) concentrations of dissolved, ionic trace metals in water bodies of the Quesnel Basin during the summer sampling period, June 23-30, 2016. Site pairs are listed together and have the same shading. Samples below detection limit are listed "DBL", and samplers that were lost or invalid are listed "NA".

	Summer DGT Trace Metal Concentrations (μ/L)						
Site	Cu	Fe	As	Se	Pb	Mn	V
Quesnel Lake at Abbott Creek	NA	NA	NA	NA	NA	NA	NA
Abbott Creek	BDL	20.5	0.059	0.007	BDL	3.76	0.326
Quesnel Lake at Whiffle Creek	1.36	4.56	0.074	0.009	BDL	1.28	0.382
Whiffle Creek	2.47	10.5	0.068	0.010	BDL	2.38	0.506
Quesnel Lake at Winkley Creek	BDL	0.619	0.060	0.005	BDL	1.28	0.187
Winkley Creek	NA	NA	NA	NA	NA	NA	NA
Polley Lake	0.384	3.83	0.517	0.042	BDL	0.964	0.187
Frypan Lake	BDL	0.234	0.031	BDL	BDL	0.428	0.869
Quesnel Lake at Raft Creek	1.90	BDL	0.034	0.003	BDL	1.10	0.113
Raft Creek	1.16	0.188	0.187	BDL	BDL	0.314	1.64
Quesnel Lake at Cedar Creek	0.136	BDL	0.179	0.006	BDL	1.13	0.374
Cedar Creek	0.547	11.9	1.12	0.010	BDL	0.921	0.476
Quesnel River	2.11	13.9	0.125	0.004	BDL	1.70	0.200
Cariboo River	0.101	43.7	0.134	0.001	0.067	2.96	0.058